Clinical Infectious Diseases

MAJOR ARTICLE

An Outpatient Clinic as a Potential Site of Transmission for an Outbreak of New Delhi Metallo-β-Lactamase–producing *Klebsiella pneumoniae* Sequence Type 716: A Study Using Whole-genome Sequencing

Amélie Heinrichs,[1] Maria Angeles Argudín,[1] Ricardo De Mendonça,[1] Ariane Deplano,[1] Sandrine Roisin,[1] Magali Dodémont,[1] Julien Coussement,[1] Lorenzo Filippin,[2] Jill Dombrecht,[3] Katrien De Bruyne,[3] Te-Din Huang,[4] Philip Supply,[5] Baudouin Byl,[6,7] Youri Glupczynski,[4] and Olivier Denis[1,6]

[1]Laboratoire de Microbiologie, CUB-Hôpital Erasme, Université Libre de Bruxelles, Brussels, [2]Laboratoire de Biologie Clinique, EpiCURA Hospital, Baudour, [3]Applied Maths-bioMérieux, Sint-Martens-Latem, [4]Laboratory of Clinical Microbiology, Belgian National Reference Center for Monitoring Antimicrobial Resistance in Gram-negative Bacteria, CHU UCL Namur, Yvoir, Belgium; [5]INSERM U1019, CNRS-UMR 8204, Center for Infection and Immunity of Lille, Institut Pasteur de Lille, Université de Lille, Lille, France; [6]Ecole de Santé Publique, Université Libre de Bruxelles, [7]Clinique d'Epidémiologie et d'Hygiène Hospitalière, CUB-Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

Background. The incidence of nosocomial infections due to carbapenem-resistant *Klebsiella pneumoniae* is increasing worldwide. Whole-genome sequencing (WGS) can help elucidate the transmission route of nosocomial pathogens.

Methods. We combined WGS and epidemiological data to analyze an outbreak of New Delhi metallo-β-lactamase (NDM)–producing *K. pneumoniae* that occurred in 2 Belgian hospitals situated about 50 miles apart. We characterized 74 NDM-producing *K. pneumoniae* isolates (9 from hospital A, 24 from hospital B, and 41 contemporary isolates from 15 other Belgian hospitals) using pulsed-field gel electrophoresis and WGS.

Results. A *K. pneumoniae* sequence type 716 clone was identified as being responsible for the outbreak with all 9 strains from hospital A and 20 of 24 from hospital B sharing a unique pulsotype and being clustered together at WGS (compared with 1 of 41 isolates from other Belgian hospitals). We identified the outpatient clinic of hospital B as the probable bridging site between the hospitals after combining epidemiological, phylogenetic, and resistome data. We also identified the patient who probably caused the transmission. In fact, all but 1 strain from hospital A carried a *Tn*1331-like transposon, whereas none of the hospital B isolates did. The patient from hospital A who did not have the *Tn*1331-like transposon was treated at the outpatient clinic of hospital B on the same day as the first NDM-producing *K. pneumoniae*–positive patient from hospital B.

Conclusions. The results from our WGS-guided investigation highlight the importance of implementing adequate infection control measures in outpatient settings, especially when healthcare delivery moves from acute care facilities to outpatient clinics.

Keywords. nosocomial outbreak; carbapenemase; infection control; whole-genome MLST; ambulatory care.

Klebsiella pneumoniae is a leading nosocomial pathogen, which is well recognized as a major public health problem [1–3]. *K. pneumoniae* isolates easily acquire and transmit determinants of antimicrobial resistance, including extended-spectrum β-lactamases and carbapenemases. In particular, the spread of carbapenemase-producing *K. pneumoniae,* largely driven by mobile genetic elements carrying carbapenemase genes, is of particular concern [4].

New Delhi metallo-β-lactamase (NDM) is an Ambler class B carbapenemase that efficiently hydrolyzes carbapenems and most β-lactams, with the exception of aztreonam [5]. NDM-producing *K. pneumoniae* isolates have disseminated worldwide and are endemic in some areas of the world, including the Indian subcontinent [4]. Multiple nosocomial outbreaks of NDM-producing *K. pneumoniae* have been reported in Asia, Europe and America [4, 6–16]. NDM-producing *K. pneumoniae* isolates responsible for outbreaks may belong to various sequence types (STs). ST11, ST15, and ST17 in particular are frequently associated with multidrug resistance [6, 7, 9, 11, 16–18].

Powerful molecular typing techniques are important to enable rapid recognition of nosocomial outbreaks caused by multidrug-resistant organisms (MDROs), and to enable strategies to be implemented to prevent the transmission of the involved pathogens [19, 20]. Whole-genome sequencing (WGS) offers many advantages for investigation of such outbreaks, including the resolution power to explore the route and direction of transmission [21–23]. However, the analysis of WGS-based typing results is still challenging and requires carefully calibrated parameters for accurate interpretation.

Received 5 March 2018; editorial decision 4 July 2018; accepted 11 July 2018; published online July 18, 2018.

Correspondence: A. Heinrichs, Laboratoire de Microbiologie, CUB-Hôpital Erasme, Université Libre de Bruxelles, 808 Rte de Lennik, 1070 Brussels, Belgium (amelie.heinrichs@ulb.ac.be).

Clinical Infectious Diseases® 2019;68(6):993–1000

DOI: 10.1093/cid/ciy581

Between 2012 and 2016, the Belgian national reference center (NRC) for antibiotic-resistant gram-negative bacilli noted an increase in the number of NDM-producing *K. pneumoniae* isolates. Currently, NDM is the second most prevalent carbapenemase among isolates referred and confirmed as carbapenemase-producing Enterobacteriaceae by our NRC [24]. In particular, 2 Belgian hospitals had outbreaks of NDM-producing *K. pneumoniae* in 2014–2015. In this study, we analyzed these NDM-producing *K. pneumoniae* outbreaks using a unique combination of WGS-based approaches to identify the link and direction of transmission between the hospitals.

METHODS

Hospital Settings

Hospital A is an academic tertiary care center of 864 beds including 36 intensive care beds divided into 5 intensive care units (ICU). Hospital A has about 30 000 admissions per year, of which 1000 are to the ICU. Hospital B is a general 211-bed institution (including 9 ICU beds) and is part of a multisite general healthcare institution of 838 beds. About 6000 patients are admitted each year to hospital B. Hospitals A and B are located in 2 different Belgian regions and are situated approximately 50 miles apart.

Definitions and Data Collection

An outbreak case was defined as a patient colonized or infected by carbapenem-nonsusceptible *K. pneumoniae* between August 2014 and December 2015. Post hoc, we classified outbreak cases as proven (NDM-producing *K. pneumoniae* of identical pulsed-field gel electrophoresis [PFGE] profile and ST) or possible (carbapenem-nonsusceptible *K. pneumoniae* but without molecular NDM detection or typing analysis). A sporadic case was defined as a molecularly identified NDM-producing *K. pneumoniae* strain not belonging to the same PFGE type and ST as the proven outbreak case strains. For each hospital, the incidence rate, expressed as the number of cases per 100 000 patient-days, was compared between the outbreak period and 1 year earlier. In addition, we collected the history of the hospital stay, the history of transfers between study hospitals, and the date and type of sample providing the first evidence of carbapenem-nonsusceptible *K. pneumoniae*. Colonization was defined as the carriage of carbapenem-nonsusceptible *K. pneumoniae* not treated with active antibiotics, whereas patients treated with active antibiotics were considered infected.

Screening Policy

K. pneumoniae isolates were collected from inpatients of the 2 study hospitals, using cultures of either clinical or rectal screening swab samples. At the time of the outbreak, a screening strategy for gram-negative MDROs was applied systematically at patient admission in 4 high-risk departments (ICU, hematology, geriatric, and gastroenterology wards) in hospital A. In addition, weekly surveillance was carried out during the hospital stay in the ICU and the Department of Hematology. Contact precautions were preemptively used in high-risk patients (ie, those with a travel history and/or previously hospitalized in another institution) until rectal swab sample culture results. This screening policy was continued in hospital A during the study period without additional measures (ie, no cohorting with dedicated nursing staff).

In hospital B, there was no systematic active screening surveillance for gram-negative MDROs until the detection of 3 patients positive for NDM-producing *K. pneumoniae* in the Department of Internal Medicine. From that moment on, the infection control team started screening for MDROs among (1) contact patients (ie, patients sharing the same room as possible outbreak cases) and (2) inpatients staying ≥15 days in hospital B. In parallel, inpatients from all departments of hospital B were screened during 3 point-prevalence surveys. Patients with positive results were placed on contact precautions and were cohorted in a single ward with dedicated nursing staff.

Bacterial Isolates and Conventional Microbiology Techniques

A total of 74 NDM-producing *K. pneumoniae* isolates were analyzed in this study (Figure 1). This included 9 isolates from hospital A and 24 from hospital B. For comparison, we also included 41 NDM-producing *K. pneumoniae* isolates collected elsewhere in Belgium and sent to the NRC in 2014 or 2015. Antimicrobial susceptibility testing was carried out using disk diffusion method (BioRad), according to the European Committee on Antimicrobial Susceptibility Testing recommendations (http://www.eucast.org/). The presence of bla_{NDM} was confirmed by means of polymerase chain reaction, as described elsewhere [25]. Molecular typing was performed by genomic macrorestriction (*Xba*I) followed by PFGE [26].

WGS, De Novo Assembly, and Genome Annotation

Nucleic acids were extracted using the DNA extraction ultraclean microbial DNA isolation kit (MOBIO). DNA libraries were prepared using Nextera XT DNA library preparation kit (Illumina), following the manufacturer's recommendations except for the normalization step, which was done manually. WGS was performed using the Illumina MiSeq platform (Illumina) to generate 200–base pair paired-end reads. Runs were accepted according to the criteria defined by Illumina for the MiSeq Reagent kit (version 3). De novo assembly was performed using the SPAdes algorithm [27]. Genomes were annotated using BioNumerics software (version 7.6; Applied Maths), and refined using the basic local alignment search tool (BLAST) when necessary (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic Analysis and Identification of Antibiotic Resistance Genes

STs were assigned in silico by uploading assembled genomes to the multilocus sequence typing (MLST) 1.8 tool (Center

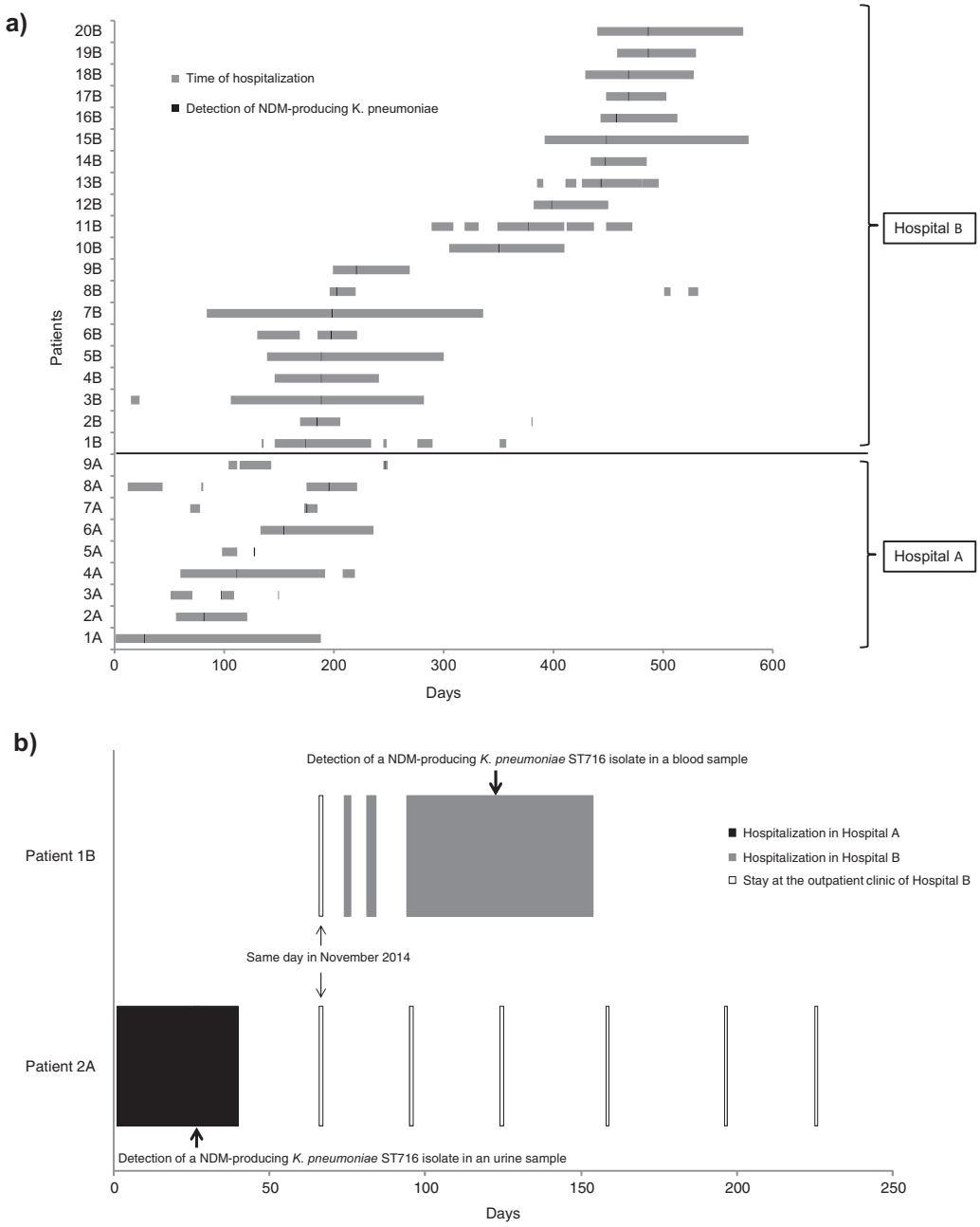

Figure 1. *a,* Timeline of detection of New Delhi metallo-β-lactamase (NDM)–producing *Klebsiella pneumoniae* in proven outbreak cases of hospitals A and B. Patients are ordered according to the date of detection of NDM-producing *K. pneumoniae* isolate (*black line*). For each patient, the time from hospital admission to discharge is represented as a gray box. *b,* Timeline regarding probable transmission from patient 2A to patient 1B via hospital B's outpatient clinic. For each patient, the hospital stay is represented as a box, with colors and sizes depending on exact location (hospital A, hospital B, or outpatient clinic of hospital B) and length of stay, respectively. Abbreviation: ST, sequence type.

for Genomic Epidemiology; http://www.genomicepidemiology.org/) [28]. The whole-genome MLST (wgMLST) *K. pneumoniae* scheme available on BioNumerics (version 7.6) was used to compare 4015 genes located on the core and accessory genome [29]. The whole-genome single-nucleotide polymorphism (SNP) analysis was done on all strains from proven outbreak cases, using Bionumerics software (version 7.6) [29]. For this purpose, we selected a de novo assembled reference genome based on sequence quality criteria for mapping, to determine SNP differences. Default filtering procedures were used to remove artifactual SNPs [30] and to construct minimum spanning trees (see Supplementary Data for additional details regarding SNP analysis). Acquired antimicrobial-resistant genes were identified by uploading

assembled genomes with ResFinder software (version 2.1; Center for Genomic Epidemiology) [31]. BLAST analysis and homemade polymerase chain reaction were used to resolve discrepancies.

Ethics

Ethical approval for this study was granted by the Ethical Committee of Université Libre de Bruxelles-Erasme Hospital (approval No. P2015/546).

RESULTS

Description of Outbreaks

The timeline of proven outbreak cases from hospitals A and B is shown in Figure 1A and Supplementary Figure 1. Metadata related to proven outbreak cases of hospitals A and B are provided in Supplementary Data (Supplementary Table 1).

Hospital A

The incidence rate of NDM-producing *K. pneumoniae* increased from a baseline rate of 1.5 cases per 100 000 patient-days (time period, August 2013 to July 2014) to 9.4 per 100 000 patient-days during the outbreak period (August 2014 to March 2015). The first patient (patient 1A) was detected in August 2014, 1 month after being admitted to the ICU. We suspected nosocomial acquisition in this first patient because he had had multiple culture-negative rectal swab specimens since admission to hospital A. However, we did not detect other concomitant NDM carrier(s) in the ICU, despite weekly surveillance screening cultures of all ICU patients. Patient 1A was then transferred to the Department of Pneumology. There, a second patient was detected with nosocomial acquisition of NDM-producing *K. pneumoniae* (patient 2A). We later identified 7 additional infected (n = 4) or colonized (n = 3) patients in 5 wards of hospital A. All 9 isolates showed the same PFGE types and belonged to ST716 (considered as proven outbreak cases). We failed to identify a clear epidemiological link to explain the acquisition of this NDM-producing *K. pneumoniae* clone for the 7 latter outbreak cases.

Hospital B

An outbreak was declared at hospital B in January 2015 and persisted until December 2015 (end of study). The incidence rate of NDM-producing *K. pneumoniae* increased from 1 case per 100 000 patient-days in 2014) to 102 cases per 100 000 patient-days in 2015. After the identification of the first 3 NDM-positive *K. pneumoniae* patients, an active surveillance screening program was implemented, as previously described (Methods section, screening policy paragraph). This demonstrated 64 additional possible outbreak cases involving the entire hospital. The most affected wards were the ICU, the Orthopedic Surgery Unit and the Departments of Geriatric Medicine and Internal Medicine. Twenty-one percent of these patients (18 of 67) were infected; the others were colonized. From the 67 possible outbreak cases, 24 isolates were available for WGS analysis, which demonstrated that 20 of 24 were proven outbreak cases (Figure 2). Of these 20 patients, 15 were colonized.

Epidemiological Link Between Hospitals A and B

From the epidemiological data, patient 2A was considered the probable link between the hospitals. First, he was repeatedly seen at the outpatient clinic of hospital B after discharge from hospital A (Figure 1B). None of the other proven outbreak case patients from hospital A were referred to hospital B. Second, the first patient detected from hospital B (patient 1B) was treated at the outpatient clinic of hospital B on the same day as patient 2A (Figure 1B). No other relevant link could be established between patients from hospitals A and B.

Population Genomic Analysis

Molecular typing using MLST, PFGE, and wgMLST enabled us to separate out the 74 NDM-producing *K. pneumoniae* strains investigated in this study, as indicated in Figure 2 (see Supplementary Data for WGS assembly quality results, Supplementary Table 2). In fact, 30 of 74 strains (ie, all 9 strains from hospital A, 20 of 24 from hospital B, and 1 of 41 from the NRC collection) were assigned to ST716, shared the same PGFE type, and clustered together in the wgMLST analysis (Figure 2). These 30 strains were closely related; they were separated by a mean pairwise allelic distance of only 17 alleles (of 4015 alleles analyzed) and by ≤6 SNPs (Figure 3). After construction of a phylogenetic tree (ie, minimum spanning tree), the strain genome from patient 2A was consistent with a progenitor status relative to hospital B cases (Figure 3). In fact, this strain was just a single SNP away from a genome type occupying a central node position relative to all hospital B genomes, and only 3 SNPs different from the patient 1B strain genome.

Of note, the single strain from the NRC (isolated in November 2015) was detected in a hospital located close to hospital B, and belonged to a patient who had been hospitalized in hospital B in October 2015. In contrast, the remaining 44 strains from hospital B (4 of 24) and the NRC (40 of 41) showed completely different pulsotypes, displayed a variety of distinct STs (ST11, ST15, ST307, and ST432) and a mean pairwise allelic distance of 3348 relative to the proven outbreak strains (in line with the allelic diversity described elsewhere for *K. pneumoniae* [32]).

Antimicrobial Susceptibility Testing and Resistome Analysis

Phenotypically, all proven outbreak strains were resistant to all tested β-lactams, fluoroquinolones and trimethoprim-sulfamethoxazole. Most were susceptible to colistin (29 of 30), chloramphenicol (26 of 30), amikacin (22 of 30), and tigecycline (18 of 30).

Our population of proven outbreak strains had 2 major phenotypic and genotypic resistance patterns (Supplementary Table 3). The first pattern (8 of 30 strains), phenotypically characterized by amikacin-resistance and genotypically by carriage of bla_{OXA-9}, *aac(6´)Ib*, and *aadA1* genes (by a *Tn*1331-like transposon), was found in all patients from hospital A, except patient

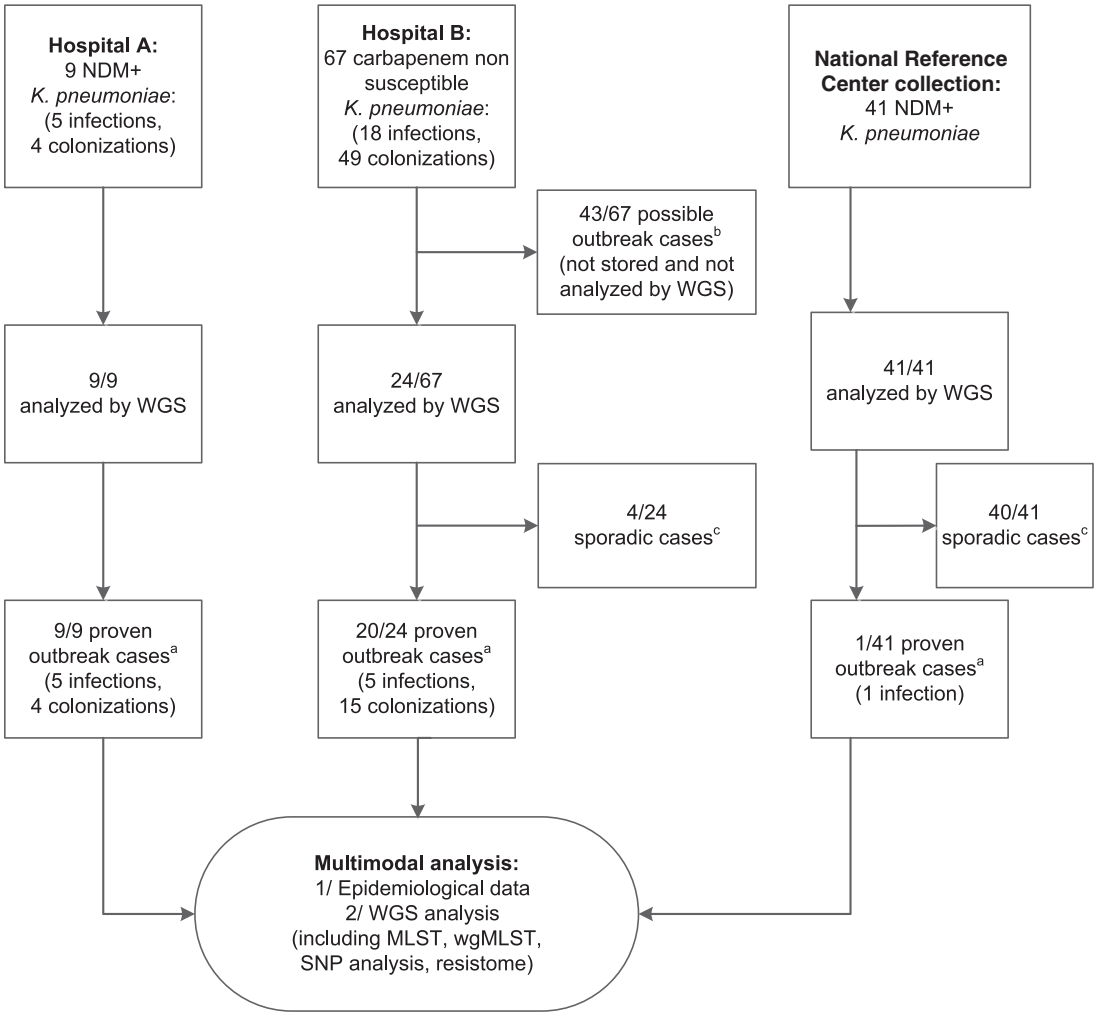

Figure 2. Flowchart of included cases and analysis performed. Proven outbreak cases were defined as colonization or infection with a New Delhi metallo-β-lactamase (NDM)–producing *Klebsiella pneumoniae* of identical pulsed-field gel electrophoresis (PFGE) type and sequence type (ST); possible outbreak cases, as colonization or infection with a carbapenem-resistant *K. pneumoniae* with no molecular analysis of the isolate; and sporadic cases, as carriage of a molecularly identified NDM-producing *K. pneumoniae* strain not belonging to the same PFGE type and ST as the proven outbreak case strains. Abbreviations: MLST, multilocus ST; NDM+, NDM-producing; SNP, single-nucleotide polymorphism; wgMLST, whole-genome MLST; WGS, whole-genome sequencing.

2A. The second pattern (22 of 30 strains) was characterized phenotypically by susceptibility to amikacin and genotypically by the absence of the previously listed genes, due to the loss of the *Tn*1331-like element. This pattern was identified in the strain from patient 2A (hospital A), as well as in all 20 proven outbreak strains collected from hospital B. We therefore concluded that patient 2A probably introduced the ST716 NDM-producing *K. pneumoniae* into hospital B.

DISCUSSION

In this study, we investigated 2 outbreaks of NDM-producing *K. pneumoniae* occurring in 2 Belgian hospitals. First, we identified *K. pneumoniae* ST716, an infrequently reported clone [33], as being responsible for both outbreaks. We combined epidemiological data with WGS data—including findings from high-resolution typing methods and a resistome analysis—to identify the outpatient clinic of hospital B as the probable transmission bridge between these outbreaks.

Because of decreasing costs and technological improvements enabling fast and high-resolution results, WGS is increasingly being used in clinical microbiology, especially to analyze nosocomial outbreaks [23]. Unlike conventional typing methods, such as PFGE and MLST, WGS provides multiple information levels from a single technique, including data on antimicrobial resistance, genome comparison, wgMLST, and SNP analysis [23].

Using PFGE and WGS, we showed that the NDM-producing *K. pneumoniae* outbreaks observed in hospitals A and B were due to the same NDM-producing *K. pneumoniae* clone (ie, ST716). The first outbreak case was detected in hospital A in August 2014. It is probable that additional

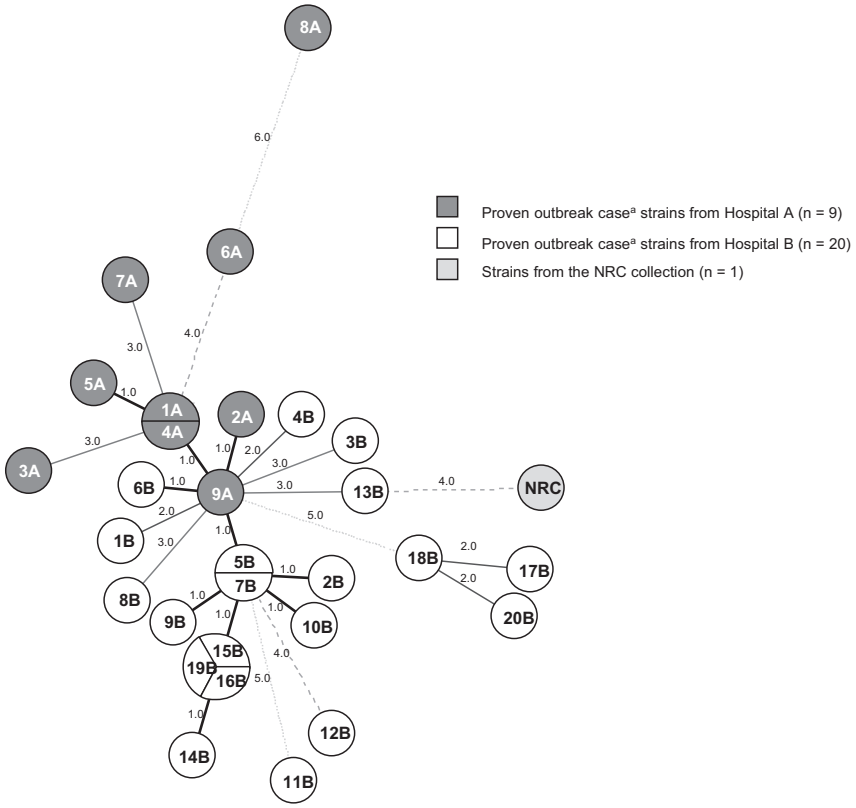

Figure 3. Minimum spanning tree based on whole-genome-single-nucleotide polymorphism analysis of the New Delhi metallo-β-lactamase (NDM)–producing *Klebsiella pneumoniae* isolates from the proven outbreak cases at hospital A (n = 9), hospital B (n = 20), and the Belgian national reference center (NRC) for antibiotic-resistant gram-negative bacilli (n = 1). Each circle represents an isolate belonging to a proven outbreak case. The codes in the circles refer to case numbers and are ordered chronologically. The numbers on the branches indicate the number of single-nucleotide polymorphisms. Proven outbreak cases are defined as colonization or infection with an NDM-producing *K. pneumoniae* of identical pulsed-field gel electrophoresis type and sequence type.

colonized patients passed unnoticed because of the lack of a systematic active surveillance screening policy for MDROs in some units and wards of hospital A. Regarding hospital B, the NDM-producing *K. pneumoniae* outbreak was detected in January 2015 and involved 67 possible outbreak cases. Our findings highlight the advantages of centralizing data (eg, at the NRC) to help document MDRO outbreaks and identify links between hospitals [34].

Our genomic and epidemiological data converged to identify the outpatient clinic of hospital B as the specific site linking the 2 outbreaks. The second proven outbreak case patient (patient 2A) was referred from hospital A to the outpatient clinic of hospital B, and his visit there overlapped with a visit by patient 1B. This epidemiological suspicion was supported by 2 distinct lines of WGS-derived evidence. First, our SNP-based phylogenetic reconstruction showed that a single SNP separated the genome of patient 2A's strain from a central node (patient 9A's strain) relative to all hospital B nodes, and patient 2A's strain genome was only 3 SNPs away from that in patient 1B. This position is consistent with the hypothesis that patient 2A carried a progenitor—or a microvariant of this progenitor—of the hospital B strains.

Second, resistome analysis allowed us to reinforce the hypothesis that the transmission occurred between patients 2A and 1B. Indeed, the loss of the *Tn*1331-like transposon detected in the patient 2A strain represents more precise evidence for the transmission from hospital A to hospital B via this patient. Patient 2A was the only patient from hospital A whose strain did not carry this transposon. Conversely, this transposon was absent in all patient strains from hospital B. The loss of this transposon served as a marker of transmission from hospital A to hospital B, in addition to MLST, wgMLST, and SNP results. Hitherto, these different layers have rarely been used in conjunction for outbreak investigation. Our study highlights the importance of a multimodal approach combining multiple WGS results (eg, findings from MLST, wgMLST, SNP analysis, and resistome data) with epidemiological data for outbreak investigation.

The hospitalization report for patient 2A did not specify his NDM-producing *K. pneumoniae* carrier status at the time of referral to the outpatient clinic at hospital B. Communication between healthcare facilities should be optimized by using electronic medical records and possibly by educating the patient [35]. Such measures may enable earlier appropriate infection

control measures to be implemented after the interfacility transfer of patients carrying MDROs. In this case, the fact that the source patient (patient 2A) was seen at an outpatient clinic rather than in an acute care facility may have facilitated the dissemination of the NDM-producing *K. pneumoniae* ST716 clone. Indeed, outpatient clinics often lack appropriate resources and strategies to prevent dissemination of MDROs, as highlighted elsewhere [36]. The implementation of adequate infection control measures in outpatient settings is crucial, because the healthcare delivery system increasingly switches between acute care facilities and outpatient clinics [35, 36].

This study has some limitations. The transmission route that we suggest (ie, transmission from patient 2A to patient 1B via the outpatient clinic of hospital B) cannot be formally proved, because no screening policy was performed before the first case was detected in hospital B. Alternate or additional transmission source(s) (eg, shared staff or visiting relatives) cannot be excluded owing to the retrospective study design. In particular, there was no systematic screening for NDM-producing *K. pneumoniae* isolates in the contact patients of outbreak cases from hospital A. As a consequence, we may have missed identifying additional carriers of this NDM-producing ST716 *K. pneumoniae* clone who were transferred to hospital B. However, both patient stay information and resistome analysis using WGS strongly support the role of patient 2A in the dissemination of the NDM-producing ST716 *K. pneumoniae* clone from hospital A to B via the outpatient clinic.

In conclusion, a multimodal analysis (ie, combination of multiple results from WGS with epidemiological data) enabled us to conclude that a multihospital outbreak of NDM-producing *K. pneumoniae* was most likely due to the same NDM-producing *K. pneumoniae* ST716 clone. More specifically, we identified the outpatient clinic as the probable transmission site bridging the 2 outbreaks. Our findings highlight the importance of implementing adequate infection control measures in outpatient settings, especially as healthcare delivery moves from acute care facilities to outpatient clinics. Further studies are required to determine the importance of outpatient facilities in the dissemination of MDROs, and possibly to identify measures to prevent the spread of these organisms in such settings.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Louise Nienhaus for molecular testing; Claire Nonhoff, Pascal Buidin, Geneviève Hay, Nathalie Legros, and Christine Thiroux for assistance in phenotypic testing; and Karen Pickett, MD for her editorial suggestions. We would like to thank all Belgian microbiology laboratories for sending resistant *K. pneumoniae* isolates to the National Reference Center for Monitoring Antimicrobial Resistance in Gram-negative Bacteria.

Financial support. This work was supported by the Bourse du Fonds Erasme Pour la Recherche Médicale (Belgium).

Potential conflicts of interest. P. S. reports consultancy fees from Genoscreen. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother **2015**; 59:5873–84.
2. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev **1998**; 11:589–603.
3. Holt KE, Wertheim H, Zadoks RN, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci U S A **2015**; 112:E3574–81.
4. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol **2016**; 7:895.
5. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in gram-negative bacteria. Biomed Res Int **2014**; 2014:249856.
6. Chung The H, Karkey A, Pham Thanh D, et al. A high-resolution genomic analysis of multidrug-resistant hospital outbreaks of *Klebsiella pneumoniae*. EMBO Mol Med **2015**; 7:227–39.
7. Zhang X, Li X, Wang M, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* causing neonatal infection in a teaching hospital in mainland China. Antimicrob Agents Chemother **2015**; 59:4349–51.
8. Khajuria A, Praharaj AK, Kumar M, Grover N, Aggarwal A. Multidrug resistant NDM-1 metallo-beta-lactamase producing *Klebsiella pneumoniae* sepsis outbreak in a neonatal intensive care unit in a tertiary care center at central India. Indian J Pathol Microbiol **2014**; 57:65–8.
9. Baraniak A, Izdebski R, Fiett J, et al. NDM-producing Enterobacteriaceae in Poland, 2012-14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. J Antimicrob Chemother **2016**; 71:85–91.
10. Seara N, Oteo J, Carrillo R, et al. Interhospital spread of NDM-7-producing *Klebsiella pneumoniae* belonging to ST437 in Spain. Int J Antimicrob Agents **2015**; 46:169–73.
11. Voulgari E, Gartzonika C, Vrioni G, et al. The Balkan region: NDM-1-producing *Klebsiella pneumoniae* ST11 clonal strain causing outbreaks in Greece. J Antimicrob Chemother **2014**; 69:2091–7.
12. Poirel L, Yilmaz M, Istanbullu A, et al. Spread of NDM-1-producing Enterobacteriaceae in a neonatal intensive care unit in Istanbul, Turkey. Antimicrob Agents Chemother **2014**; 58:2929–33.
13. Gaibani P, Ambretti S, Berlingeri A, et al. Outbreak of NDM-1-producing Enterobacteriaceae in northern Italy, July to August 2011. Euro Surveill **2011**; 16:20027.
14. Borgia S, Lastovetska O, Richardson D, et al. Outbreak of carbapenem-resistant Enterobacteriaceae containing *bla*NDM-1, Ontario, Canada. Clin Infect Dis **2012**; 55:e109–17.
15. Escobar Pérez JA, Olarte Escobar NM, Castro-Cardozo B, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. Antimicrob Agents Chemother **2013**; 57:1957–60.
16. Stoesser N, Giess A, Batty EM, et al. Genome sequencing of an extended series of NDM-producing *Klebsiella pneumoniae* isolates from neonatal infections in a Nepali hospital characterizes the extent of community- versus hospital-associated transmission in an endemic setting. Antimicrob Agents Chemother **2014**; 58:7347–57.
17. Zhou K, Lokate M, Deurenberg RH, et al. Use of whole-genome sequencing to trace, control and characterize the regional expansion of extended-spectrum β-lactamase producing ST15 *Klebsiella pneumoniae*. Sci Rep **2016**; 6:20840.
18. Jin Y, Shao C, Li J, Fan H, Bai Y, Wang Y. Outbreak of multidrug resistant NDM-1-producing *Klebsiella pneumoniae* from a neonatal unit in Shandong Province, China. PLoS One **2015**; 10:e0119571.
19. Sabat AJ, Budimir A, Nashev D, et al; ESCMID Study Group of Epidemiological Markers (ESGEM). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill **2013**; 18:20380.
20. Mellmann A, Bletz S, Böking T, et al. Real-time genome sequencing of resistant bacteria provides precision infection control in an institutional setting. J Clin Microbiol **2016**; 54:2874–81.
21. Ruppé E, Olearo F, Pires D, et al. Clonal or not clonal? investigating hospital outbreaks of KPC-producing *Klebsiella pneumoniae* with whole-genome sequencing. Clin Microbiol Infect **2017**; 23:470–5.

22. Croucher NJ, Didelot X. The application of genomics to tracing bacterial pathogen transmission. Curr Opin Microbiol **2015**; 23:62–7.
23. Quainoo S, Coolen JPM, van Hijum SAFT, et al. Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. Clin Microbiol Rev **2017**; 30:1015–63.
24. Huang TD, Bogaerts P, Berhin C, Bauraing C, Jans B, Glupczynski Y. Microbiological surveillance of carbapenemase-producing Enterobacteriaceae in Belgium in 2016. In: 27th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID); Vienna, Austria; 22–25 April **2017**. P0409.
25. Bogaerts P, Rezende de Castro R, de Mendonça R, Huang TD, Denis O, Glupczynski Y. Validation of carbapenemase and extended-spectrum β-lactamase multiplex endpoint PCR assays according to ISO 15189. J Antimicrob Chemother **2013**; 68:1576–82.
26. Laurent C, Rodriguez-Villalobos H, Rost F, et al. Intensive care unit outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* controlled by cohorting patients and reinforcing infection control measures. Infect Control Hosp Epidemiol **2008**; 29:517–24.
27. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **2012**; 19:455–77.
28. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol **2012**; 50:1355–61.
29. BioNumerics release note: *Klebsiella pneumoniae* schema for whole genome sequencing typing. Available at: http://www.applied-maths.com/sites/default/files/extra/Release-Note-Klebsiella-pneumoniae-schema.pdf. Accessed 22 January 2018.
30. Pouseele H, Supply P. Accurate whole-genome sequencing-based epidemiological surveillance of *Mycobacterium tuberculosis*. In: Sails AD, Tang YW. Current and emerging technologies for the diagnosis of microbial infections. Methods in Microbiology, vol 42: Amsterdam, the Netherlands: Elsevier, **2015**:359–94.
31. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother **2012**; 67:2640–4.
32. Bialek-Davenet S, Nicolas-Chanoine MH, Decré D, Brisse S. Microbiological and clinical characteristics of bacteraemia caused by the hypermucoviscosity phenotype of *Klebsiella pneumoniae* in Korea. Epidemiol Infect **2013**; 141:188.
33. Li B, Hu Y, Wang Q, et al. Structural diversity of class 1 integrons and their associated gene cassettes in *Klebsiella pneumoniae* isolates from a hospital in China. PLoS One **2013**; 8:e75805.
34. Tacconelli E, Sifakis F, Harbarth S, et al. Surveillance for control of antimicrobial resistance. Lancet Infect Dis **2018**; 18:e99–106.
35. Flanagan E, Cassone M, Montoya A, Mody L. Infection control in alternative health care settings: an update. Infect Dis Clin North Am **2016**; 30:785–804.
36. Centers for Disease Control and Prevention. Guide to infection prevention for outpatient settings: minimun expectations for safe care. Available at: https://www.cdc.gov/hai/settings/outpatient/outpatient-care-guidelines.html. Accessed 29 November 2017.