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Major Article

Stalking a lethal superbug by whole-genome sequencing and phylogenetics: Influence on unraveling a major hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae*

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Background: From July 2010-April 2013, Leipzig University Hospital experienced the largest outbreak of a *Klebsiella pneumoniae* carbapenemase 2 (KPC-2)-producing *Klebsiella pneumoniae* (KPC-2-*Kp*) strain observed in Germany to date. After termination of the outbreak, we aimed to reconstruct transmission pathways by phylogenetics based on whole-genome sequencing (WGS).

Methods: One hundred seventeen KPC-2-*Kp* isolates from 89 outbreak patients, 5 environmental KPC-2-*Kp* isolates, and 24 *K pneumoniae* strains not linked to the outbreak underwent WGS. Phylogenetic analysis was performed blinded to clinical data and based on the genomic reads.

Results: A patient from Greece was confirmed as the source of the outbreak. Transmission pathways for 11 out of 89 patients (12.4%) were plausibly explained by descriptive epidemiology, applying strict definitions. Five of these and an additional 15 (ie, 20 out of 89 patients [22.5%]) were confirmed by phylogenetics. The rate of phylogenetically confirmed transmissions increased significantly from 8 out of 66 (12.1% for the time period before) to 12 out of 23 patients (52.2% for the time period after; $P < .001$) after implementation of systematic screening for KPC-2-*Kp* (33,623 screening investigations within 11 months). Using descriptive epidemiology, systematic screening showed no significant effect (7 out of 66 [10.6%] vs 4 out of 23 [17.4%] patients; $P = .465$). The phylogenetic analysis supported the assumption that a contaminated positioning pillow served as a reservoir for the persistence of KPC-2-*Kp*.

Conclusions: Effective phylogenetic identification of transmissions requires systematic microbiologic screening. Extensive screening and phylogenetic analysis based on WGS should be started as soon as possible in a bacterial outbreak situation.

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TK, KF, ACR, JT, and CL participated in the study conception and design. TK, SF, SB, and CL were responsible for patient identification and acquisition of clinical data. SB, UXK, and CL performed the clinical evaluation of study patients. TK, MH, and NL carried out the major laboratory experiments. TK, KF, MK, TE, SB, ACR, JT, and CL analyzed the data. CL wrote the manuscript. All authors read and approved the final version.

TK and KF contributed equally as first authors.

ACR, JT, and CL contributed equally as senior authors.

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Klebsiella pneumoniae (*Kp*) is a major cause of nosocomial infections, primarily among debilitated patients.[1] *Kp* carbapenemases (KPCs) have rapidly spread since 1996[1,2]; outbreaks in Europe are mainly associated with the bla_{KPC-2} gene and the globally dominant multilocus sequence type 258 (ST258).[2]

From July 2010-April 2013, Leipzig University Hospital (LUH) experienced the largest outbreak of a KPC-2-*Kp* strain (ST258) in Germany to date.[2,3] The outbreak was linked to a patient transferred from a hospital in Rhodes, Greece, where KPC-producing pathogens have been endemic since 2007.[2,4] A total of 105 patients were identified as being either colonized (60 out of 105 [57.1%]) or infected (45 out of 105 [42.9%]) with KPC-2-*Kp*. Significant clinical features of the outbreak have been described in previous studies.[5-10]

Genome sequencing technologies and phylogenetic analysis now allow researchers to discriminate between closely related bacterial clones of the same lineage, helping to analyze outbreak scenarios.[11] For instance, applying this method, a small outbreak of KPC-producing *Kp* at the National Institutes of Health Clinical Center in Bethesda, MD, during 2012 could be understood better and transmission pathways were discovered more easily.[12]

This article describes the phylogenetic analysis of KPC-2-*Kp* outbreak isolates based on their genomic sequences and analyzes its additional value to reconstruct transmission routes in the 34-month outbreak at LUH.

METHODS

Study design

This was a retrospective study. Eighty-nine out of 105 KPC-2-*Kp* positive outbreak patients could be included. Data were collected by medical record review.

Setting

LUH is a tertiary care facility with approximately 1,400 beds, more than 50,000 inpatients, and approximately 310,000 outpatients per year. It has an extensive transplant program involving liver, kidney, pancreas, lung, bone marrow, and peripheral blood stem cell transplantation. There is a tight exchange of patients between the gastroenterology/hepatology wards, peripheral surgical wards, and the interdisciplinary surgical intensive care unit (SICU) by the transplant program and the joint care of patients with visceral diseases.

Patients and definitions

A KPC-2-*Kp* case was defined as any hospitalized patient in whom KPC-2-*Kp* was isolated from a clinical or screening specimen between June 28, 2010, and April 2, 2013. Clinical infection was determined based on isolating KPC-2-*Kp* from a clinical specimen and a medical diagnosis, whereas colonized patients showed no clinical manifestation. We determined KPC-2-*Kp* carriage by isolating the pathogen and assessing carbapenem-resistance by applying susceptibility testing according to International Organization for Standardization guideline 20776, confirmed by KPC-2–specific polymerase chain reaction (PCR) tests.

In accordance with Centers for Disease Control and Prevention recommendations, we started active surveillance for all patients on June 1, 2012, as screening for KPC-2-*Kp* in fecal samples or rectal swabs,[13] employing CHROMagar KPC chromogenic agar plates (CHROMagar, Paris, France) and KPC-2–specific PCR tests. Cultures and PCR tests were applied as systematic screening procedures that were repeated on a weekly basis for each hospitalized patient (leading to 33,623 microbiologic screening investigations within 11 months).

Using descriptive epidemiology, KPC-2-*Kp* cases were assigned to a specific transmission chain if direct room contact with another KPC-2-*Kp*-positive case and/or documented care by the same nursing staff in the same shift could be established. Applying phylogenetics, cases were considered to belong to the same transmission chain if they were within the same phylogenetic clade and patients had direct ward contact with another KPC-2-*Kp*–positive case and/or had documented care by the same nursing staff members.

Data sources

Clinical and microbiologic data were retrieved using patients' charts and LUH's patient data management system (i.s.h.med; SAP, Walldorf, Germany).

Bacterial strain typing and sequencing

In total, 152 *Kp* isolates were available for sequencing. Six samples were excluded due to insufficient sequencing coverage (<30×). The remaining 146 samples included 117 specimens from 89 KPC-2–positive outbreak patients (66 patients with 1 isolate, 19 patients with 2 isolates, 3 patients with 3 isolates, and 1 patient with 4 isolates; all isolates were obtained within the outbreak period), 5 environmental KPC-2-*Kp* samples from the outbreak period, 7 specimens of KPC-producing *Kp* from other countries (Greece, Italy, Poland, and Brazil), 5 KPC-2–producing *Kp* isolates from surrounding hospitals, 8 *Kp* isolates without KPC-mediated resistance from LUH, and 4 *Kp* isolates from the Faculty of Veterinary Medicine, University of Leipzig. Bacterial DNA was extracted using either the DNeasy Blood Kit (Qiagen, Hilden, Germany) or the Tissue Gentra Puregene Blood Kit (Qiagen) for sequencing on the 454 platform (Roche Sequencing, Pleasanton, CA) and the HiSeq 2000 platform (Illumina, San Diego, CA), respectively, or phenol chloroform DNA extraction for sequencing on the PacBio RSII platform (Pacific Biosciences, Menlo Park, CA). DNA samples were sheared using the Bioruptor (Diagenode, Seraing, Belgium) with 5 cycles (20 seconds on and 20 seconds off), producing adequate fragment lengths with an average library size of 650 bp. Shearing was not applied for samples regarding long read sequencing.

In silico multilocus sequence typing of individual KPC-2-*Kp* outbreak isolates (based on polymorphisms of *rpoB, gapA, mdh, pgi, phoE, infB*, and *tonB* genes) was performed as described elsewhere.[14]

Sequencing the reference genome

The first detected KPC-2-*Kp* isolate from the patient transferred from Rhodes, Greece, was used to sequence the reference genome. In a first strategy, DNA samples were sequenced with PacBio RSII, which supports modes that generate circular consensus sequencing short reads and long reads. Circular consensus sequencing reads were collapsed into consensus read sequences and assembled with long reads into contigs using Pacific Bioscience's proprietary software. This returned 131,138 consensus reads (average read length, 546 bp and maximum read length, 2,567 bp). All reads were assembled into 125 contigs (average length, 46,232 bp and maximum length, 482,734 bp). In the second strategy, an aliquot of the same DNA sample was sequenced on the 454 platform. Quality filtered reads were assembled into contigs using 454's proprietary software Newbler (Roche Sequencing) with default settings. The assembly returned 1,070 contigs (average size, 5,414 bp and maximum size, 49,594 bp). Contigs from both approaches were further assembled using Minimus2 (https://sourceforge.net/projects/amos/). All contigs were identified as either of genomic origin or plasmids