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Investigation of a suspected nosocomial transmission of bla_{KPC3}-mediated carbapenem-resistant *Klebsiella pneumoniae* by whole genome sequencing

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ABSTRACT

Whole genome sequencing (WGS) was compared to pulse-field gel electrophoresis (PFGE) of XbaI-digested genomic DNA, as methods by which to evaluate a potential transmission of carbapenem-resistant *Klebsiella pneumoniae* between 2 hospital inpatients. PFGE result demonstrated only 1-band difference between the isolates, suggesting probable relatedness. In contrast, while WGS data demonstrated the same sequence type and very similar chromosomal sequences, over 20 single nucleotide variants were identified between the isolates, bringing into question whether there was a transmission event. WGS also identified an additional plasmid, with an XbaI restriction site in the isolates of the second patient that was not identified by PFGE. While WGS provided additional information that was not available by PFGE, in this study, neither method could definitively conclude the relatedness between the isolates.

1. Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) are an evolving threat to public health. At the present time in the United States, carbapenem resistance among the Enterobacteriaceae is predominantly mediated by the class A β-lactamase, KPC (Gupta et al., 2011; Munoz-Price et al., 2013; Temkin et al., 2014; Tzouvelekis et al., 2012). bla_{KPC} genes are typically encoded on plasmids that harbor resistance determinants for several other antimicrobial classes (Nordmann et al., 2011; Schultsz and Geerlings, 2012). As a result, such CRE are resistant to many, if not all, currently available antimicrobials and are extremely difficult to treat (Temkin et al., 2014; Tzouvelekis et al., 2012). The bla_{KPC}-harboring plasmids are efficiently mobilized both between isolates of the same species and across genera within the family Enterobacteriaceae, providing an opportunity for rapid dissemination of carbapenem resistance in healthcare settings (Cuzon et al., 2010; Mathers et al., 2011; Nordmann et al., 2009; Sidjabat et al., 2009). Evaluation of hospital-based outbreaks of KPC-producing *Klebsiella pneumoniae* can be challenging, as the majority of KPC-producing isolates in the United States are of a common lineage, sequence type (ST) 258 (Kitchel et al., 2009). Furthermore, KPC-producing *K. pneumoniae* can colonize the gastrointestinal tract of exposed patients for prolonged periods of time, silently introducing these isolates into healthcare facilities, and potentially only causing clinical infections weeks to months after hospital admission. Pulse-field gel electrophoresis (PFGE) remains the gold standard method used to evaluate isolates of CRE in cases of suspected intrafacility transmission (Goering, 2010). However, in regions where KPC is endemic, PFGE data may not yield the level of discrimination required to differentiate intrafacility transmission from introduction of a closely related strain from another healthcare facility. In contrast, sequencing of

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whole bacterial genomes has been shown to be a powerful tool by which to track transmission of KPC-producing isolates within healthcare facilities (Snitkin et al., 2012). Whole genome sequence (WGS) data allow not only high-level resolution of genomic differences between bacteria but investigation of antimicrobial resistance genes and their associated mobile genetic elements such as transposons and plasmids (Mathers et al., 2015c).

At our institution, the proportion of Enterobacteriaceae resistant to carbapenems is <0.5%, and KPC accounts for the carbapenem-resistant phenotype in 90% of these isolates (Pollett et al., 2014). Nearly, all patients with CRE to date have had recent history of treatment in long-term acute care hospitals and/or nursing homes, and for the vast majority of cases, isolates were recovered in the first 48 hours of hospitalization. As such, when carbapenem-resistant *K. pneumoniae* (CRKP) were isolated from 2 patients, both after prolonged hospitalization in our surgical intensive care unit (ICU), we launched an investigation to determine if these cases might represent hospital-based transmission. We compared WGS to PFGE as tools to aid in this investigation, at our facility with a low baseline incidence of CRE.

2. Materials and methods

2.1. Clinical isolates and antimicrobial susceptibility testing

Three CRKP isolates recovered from bile fluid from patient A (CRKP-A) obtained hospital day 100 and from respiratory secretions (CRKP-B1) and blood (CRKP-B2) from patient B collected hospital day 134 in 2014 were evaluated. Two additional CRKP (CRKP-X and CRKP-Y), isolated from 2 different patients hospitalized in our facility around the same time, were evaluated as controls. Antimicrobial susceptibility was performed by the Clinical and Laboratory Standards Institute reference broth microdilution method, on panels prepared in-house, as described elsewhere (Pollett et al., 2014).

2.2. PFGE analysis

PFGE analysis of the CRKP isolates was performed using an XbaI digestion of DNA from the 2 CRE isolates (CRKP-A and CRKP-B1), as previously described for *Escherichia coli* (http://www.cdc.gov/pulsenet/protocols.htm). PFGE data analysis was performed using BioNumerics V. 6.6 11 (Applied Maths, Inc., Austin, TX).

2.3. WGS

DNA was extracted from the isolates using a tissue DNA extraction kit on a BioRobot EZ1 (Qiagen, Valencia, CA, USA). Genomic shotgun libraries were generated using Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's instruction. WGS was performed using an Illumina MiSeq with a 2X250bp v2 sequencing protocol.

2.4. WGS data analysis

Between 1.1 and 1.8 million reads per sample were acquired, and de novo assembly was performed using SPAdes 3.1.1 (http://bioinf.spbau.ru/spades). For each isolate, the number of contigs ranged from 111 to 132; the largest contigs were 368 kb to 430 kb; and the total genome lengths ranged from 5.55 Mb to 5.71 Mb. Raw paired reads from Illumina MiSeq for each isolate were processed using Galaxy tools (Goecks et al., 2010) and submitted for single nucleotide variant (SNV) analysis and generation of SNV phylogenetic tree (maximum likelihood method) using Center of Genomic Epidemiology (CGE) CSI Phylogeny (Kaas et al., 2014) (https://cge.cbs.dtu.dk/services/CSIPhylogeny). The SNV calling was based on similar quality filter criteria described elsewhere (Salipante et al., 2015a): 1) a minimum of 15 reads, 2) relative depth at SNV positions of 50%, 3) minimum distance between SNVs of 10, 4) minimum SNV quality of 30, and 5) minimum read mapping quality of 25 and minimum Z-score of 1.96. Multilocus sequencing type (MLST) was identified through use of CGE MLST 1.7 (https://cge.cbs.dtu.dk/services/MLST). Antimicrobial resistance genes were identified by both RAST (http://rast.nmpdr.org) and CGE ResFinder (https://cge.cbs.dtu.dk/services/ResFinder). The contigs containing antimicrobial resistance genes were analyzed by BLAST to identify closely matched plasmids; the entire contigs were then mapped to these plasmids using CONTIGuator (Galardini et al., 2011) (http://contiguator.sourceforge.net). The closest plasmid was then used as reference sequence to perform mapping from the raw paired-end reads by using Geneious (Biomatters, Auckland, New Zealand), generating a hypothetical plasmid map based on the consensus sequence. The WGS data have been deposited in GenBank with accession nos. SAMN03997506 (CRKP-A), SAMN03997511 (CRKP-B1), and SAMN03997513 (CRKP-B2).

2.5. PFGE band DNA gel purification and Sanger sequencing analysis

The regions of the gel corresponding to the 121.6–198.2 kb size bands were cut out and DNA was recovered and purified using Zymoclean Large Fragment DNA Recovery Kit (Zymo Research, Irvine, CA, USA). Several sets of PCR primers were designed (Table 1) with 1 set amplifying a 648-bp region immediately upstream of XbaI restriction site on the pRMH760-like plasmid, 1 set amplifying a 757-bp region immediately downstream of the XbaI site, and 1 set amplifying a 735-bp region across the XbaI site. Two more primer sets were designed to amplify the region containing *aadB* gene and *aph(3′)-lb* gene present on this plasmid, respectively. PCR was performed using AmpliTaq Gold Fast PCR Master Mix (Thermo Fisher, Carlsbad, CA, USA) and visualized by conventional agarose gel electrophoresis. The same PCR primers were diluted 10-fold and used as sequencing primers. Sanger sequencing analysis was performed using BigDye Terminator v3.1 cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer (Thermo Fisher).

3. Results

3.1. Case summaries and epidemiologic investigation

Patient A had end-stage liver disease due to alcoholic cirrhosis that was complicated by ascites and renal failure. The patient was transferred to our surgical ICU following a short stay at an outside hospital for management of sepsis secondary to peritonitis and evaluation for liver transplantation. On hospital day 43, a CRKP was isolated from patient A's respiratory secretions and 57 days later from the patient's bile fluid (CRKP-A, obtained hospital day 100). Patient A was placed on contact precautions per hospital policy. Patient B had hepatitis C and alcoholic liver cirrhosis and was transferred from a long-term acute care facility after a prolonged hospitalization for complicated cholecystitis, sepsis, respiratory failure, and acute renal failure. Patient B was admitted to the same surgical ICU as patient A, 42 days after patient A's admission and the day prior to the first isolation of CRKP from patient A. After 93 days hospitalization, the first 40 of which were in a room adjacent to patient A, CRKP was isolated from patient B's abdominal drainage. Forty-one days later, CRKP-B1 (collected hospital day 134) was isolated from respiratory secretions; and CRKP-B2 (collected hospital day 134), from blood. Review of medical records and unit logs revealed several shared care personnel between the patients, including physicians, nurses, care partners, allied health workers (e.g., dialysis), and housekeeping. Additionally, both patients underwent endoscopic procedures, liver transplantation, intubation, and central line placement prior to isolation of CRKP. Due to the multitude of commonalities, we determined that there was a high likelihood of unit-based transmission of CRKP from patient A to patient B. No surveillance cultures were performed on these patients, either at admission or during their hospitalization, to evaluate for CRKP colonization.