Genomic Epidemiology of an Outbreak of *Klebsiella pneumoniae* ST471 Producing Extended-Spectrum β-Lactamases in a Neonatal Intensive Care Unit

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Purpose: *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs) causes nosocomial infections worldwide. The present study aimed to determine the molecular subtyping characteristics and antibiotic resistance mechanisms of ESBL-producing *K. pneumoniae* strains collected during an outbreak. Moreover, we attempted to reveal the fine transmission route of the strains within this outbreak using whole-genome sequencing (WGS).

Methods: Collecting cases and strain information were carried out. Outbreak-related strains were identified using pulsed-field gel electrophoresis (PFGE). The antibiotic susceptibility, drug-resistant genes, and molecular subtype characteristics of ESBL-producing *K. pneumoniae* were analyzed. The fine transmission route of the strains within this outbreak was revealed using WGS and minimum core genome (MCG) sequence typing.

Results: In mid-January, 2015, five cases of neonatal pneumonia caused by ESBL-producing *K. pneumoniae* were observed in the neonatal intensive care unit (NICU) of the Affiliated Hospital of Chifeng University, China. Eight ESBL-producing *K. pneumoniae* were isolated from these five cases, and two additional strains from another two cases were identified using PFGE. All ten isolates harbored $bla_{CTX-M-15}$, bla_{TEM-1}, $bla_{SHV-108}$, and bla_{OXA-1} genes, and belonged to the sequence type 471 (ST471) clone. A putative transmission map was constructed via comprehensive consideration of genomic and epidemiological information. WGS identified the initial case and the "superspreader". The genomic epidemiological investigation revealed that the outbreak was caused by the introduction of the bacteria one month before the first case appeared.

Conclusion: As far as we know, this is the first report to describe the characteristics of an ST471 ESBL-producing *K. pneumoniae* outbreak. The data showed that epidemiological inferences could be greatly improved by interpretation in the context of WGS and that *K. pneumoniae* strains isolated from the same outbreak contain sufficient genomic differences to refine epidemiological linkages on the basis of genetic lineage. These findings suggested that integration of genomic and epidemiological data can help us to have a clearer understanding of when and how outbreaks occur, so as to better control nosocomial transmission.

Keywords: whole-genome sequencing, minimum core genome, superspreader, transmission map

Introduction

Klebsiella pneumoniae, one of the "ESKAPE" pathogens, has emerged as an important nosocomial pathogen that mainly causes infections in the respiratory tract, urinary tract, and blood.[1,2] The prevalence of multi-drug resistant (MDR) *K. pneumoniae* has increased dramatically in recent years. MDR *K. pneumoniae*

frequently exhibits resistance to extended-spectrum cephalosporins because of its production of extended-spectrum β-lactamases (ESBLs).[3] ESBL-producing bacteria cause higher morbidity, mortality, and fiscal burden.[4,5] This limits efficient clinical treatment tremendously, resulting in undesirable treatment outcomes.

The outbreak of multidrug-resistant bacteria is a serious public health problem, and nosocomial outbreaks caused by ESBL-producing *K. pneumoniae* have been reported worldwide.[6–11] ESBL-producing *K. pneumoniae* is the most frequently implicated pathogen causing outbreaks in neonatal intensive care units (NICUs), with mortality rates up to 31% among infected infants.[12] Traditional molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), can define the outbreak strains well, but cannot reveal the transmission routes between strains.

Over the past decade, whole-genome sequencing (WGS) technology has developed rapidly and has been used widely in epidemiological investigations of bacterial infectious diseases, to identify outbreaks,[13,14] trace pathogen transmission,[15,16] discover new modes or ways of transmission,[17] identify new clones,[18] and track nosocomial infections and outbreaks.[19] In the present study we report an outbreak of *K. pneumoniae* producing ESBLs that took place in a NICU and attempt to reveal the fine transmission route of the strains within this outbreak.

Materials and Methods
Review of NICU Records and Collection of Patient Information
The outbreak occurred in the NICU of the Affiliated Hospital of Chifeng University, a 2000-bed general tertiary care and University-affiliated teaching hospital in Inner Mongolia, China. This hospital accepts 2200 outpatient and emergency patients each day. The NICU has a total of 25 beds.

The medical records of patients from whom ESBL-producing *K. pneumoniae* was isolated were reviewed, including time of stay in the NICU, bed site, clinical symptoms, diagnosis, data of the ESBL-producing *K. pneumoniae* isolation, and outcomes.

Bacterial Identification and Antimicrobial Susceptibility
Bacterial identification was initially performed using the VITEK-2 automated system (BioMérieux, Craponne, France) and further confirmed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Bruker, Leipzig, Germany).

Antibiotic susceptibility testing was performed using the broth dilution method. We measured the minimum inhibitory concentration (MIC) of ampicillin (AMP), ampicillin/sulbactam (AMS), amoxicillin/clavulanate (AMC), cefazolin (CFZ), cefoxitin (CFX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), aztreonam (AZM), imipenem (IMI), meropenem (MEM), amikacin (AMI), kanamycin (KAN), azithromycin (AZI), tetracycline (TET), doxycycline (DOX), tigecycline (TGC), ciprofloxacin (CIP), levofloxacin (LEV), trimethoprim-sulfamethoxazole (SXT), chloramphenicol (CHL), and colistin (CO). All antibiotics, except tigecycline and colistin, were interpreted according to the approved standard of Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines.[20] Susceptibility to tigecycline and colistin was defined based on the criteria proposed by the European Committee on Antimicrobial Susceptibility Testing-2018 (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf). *Escherichia coli* ATCC 25922 was used as quality control strain for the antibiotic susceptibility testing.

We screened genes for resistance to beta-lactamase (bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{PER}, bla_{VEB}) in all strains, as described previously.[21] DNA sequencing was performed on both strands of the PCR amplification products. The results were compared and aligned with reference sequences using the online BLAST database.

Pulsed-Field Gel Electrophoresis (PFGE)
We used the 1-day, standardized PFGE protocol for *K. pneumoniae*.[22] Cell suspensions were placed in polystyrene tubes (Falcon 12 × 75 mm, from Fisher Scientific, Hampton, NH, USA), and their optical density was adjusted to 3.8–4.0 using a Densimat photometer (BioMérieux). *K. pneumoniae* slices were digested using 50 U per slice of *Xba*I (Takara, Dalian, China) for 4 h at 37 °C, and electrophoresis was performed using a CHEF-DRIII system (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis was performed using a switch time of 6 s to 36 s for 18.5 h, and images were captured using a Gel Doc 2000 system (Bio-Rad) and converted to TIFF files. The TIFF files were analyzed using BioNumerics version 5.1 software (Applied Maths, Kortrijk, Belgium).

Multilocus Sequence Typing (MLST)
MLST using seven genes (*gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB*) was performed on the isolates according to the

protocol described on the *K. pneumoniae* MLST website (http://bigsdb.pasteur.fr/). Alleles and sequence types (STs) were assigned using the MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

Whole-Genome Sequencing (WGS), Detection of Single Nucleotide Polymorphisms (SNPs), and Minimum Core Genome (MCG) Sequence Typing

The ten outbreak strains were selected for WGS. Genomic DNA from the bacterial strains was sequenced using Illumina sequencing by constructing two paired-end (PE) libraries with average insertion lengths of 500 bp and 2000 bp, respectively. Sequences were generated using an Illumina GA IIx apparatus (Illumina Inc., San Diego, CA, USA). Raw data was processed in four steps, including removing reads with 5 bp of ambiguous bases, removing reads with 20 bp of low quality ($\leq$ Q20) bases, removing adapter contamination, and removing duplicated reads. Finally, 100× libraries were obtained with clean PE read data. Assembly was performed using SOAPdenovo v1.05.[23]

The whole-genome sequence of *K. pneumoniae* KPR0928 (NCBI accession number: NZ_CP008831.1) was used as the reference sequence, and clean reads of the sequenced isolates were mapped to the reference genome using bowtie 2 software under the default parameters.[24] SNPs were then identified using Samtools and combined together according to the reference.[25] SNPs with low quality reads (read depth < 5) and those located within 5 bps on the chromosome were removed to avoid the effect of recombination, as described in previous studies.[17,26] The isolates were clustered and a heatmap was generated using the heatmap

package in R. The transmission route was then reconstructed based on the emergence of different SNPs in each isolate and the case information, including the time of the onset of infection and the hospitalization time.

The multilocus sequences were identified using the pMLST 2.0 database (https://cge.cbs.dtu.dk/services/MLST/).[27] Antimicrobial resistance genes were identified by searching the Comprehensive Antibiotic Research Database (CARD, https://card.mcmaster.ca/).[28]

Nucleotide Sequence Accession Numbers

The data from the WGS project have been deposited at GenBank under the BioProject ID PRJNA578669.

Results
Overall Description of the Outbreak

In mid-January, 2015, we identified five cases (cases 1–5 in Table 1 and Figure 1) of neonatal pneumonia caused by ESBL-producing *K. pneumoniae* in the NICU of our hospital. This NICU consists of two units with a total of four wards and 25 beds, and has been open since 2013.

The index case (case 1) of this outbreak was identified on February 13, 2015. On February 16, four more cases (cases 2–5) in the same unit in the NICU were observed (Table 1). The timeline of patient admission and ESBL-producing *K. pneumoniae* isolation is shown in Figure 1. An outbreak was declared and stringent infection control measures were implemented, including contact precautions, strengthening of hand hygiene, environmental cleaning, and enhancing antimicrobial stewardship.

Table 1 Descriptions of the Five Cases Involved in the February 16th Outbreak in the NICU

| Case No.	Clinical Symptoms and Diagnosis	Time of Stay in NICU	Date of the ESBL-Producing *K. pneumoniae* Isolation	Outcome
Case 1	Pneumonia, respiratory failure, brain injury, anemia, jaundice	3/1/2015 − 2/3/2015	20/1/2015, 13/2/2015	Recovery
Case 2	Pneumonia, brain injury, anemia, jaundice, hypoproteinemia, metabolic acidosis	10/1/2015 − 24/3/2015	16/2/2015, 20/3/2015	Recovery
Case 3	Pneumonia, brain injury, jaundice, metabolic acidosis, hemangioma	25/1/2015 − 4/3/2015	16/2/2015, 2/3/2015	Recovery
Case 4	Pneumonia, brain injury, anemia, jaundice	29/1/2015 − 7/3/2015	16/2/2015	Recovery
Case 5	Pneumonia, respiratory failure, brain injury, anemia, jaundice, metabolic acidosis	31/12/2014-3/3/2015	16/2/2015	Recovery
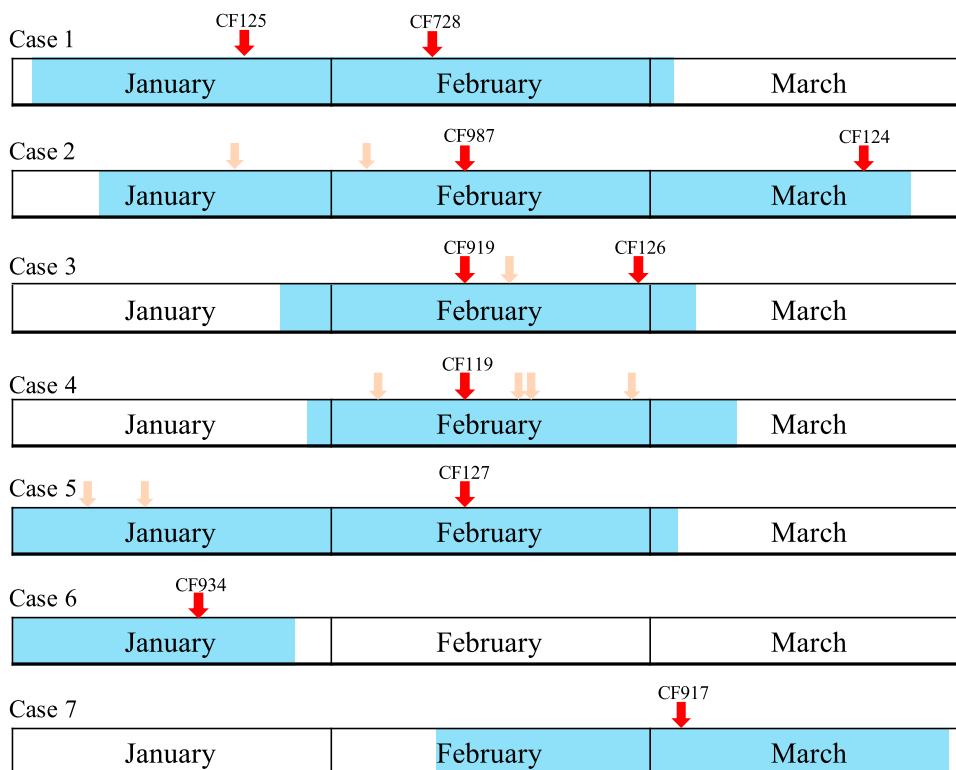
Figure 1 The timeline of patient admission and ESBL-producing *K. pneumoniae* isolation. Shadows on the timeline represent the duration of the case. Different wards are indicated in different colors. The red arrows indicate the isolation of the strains. Light red arrows represent sampling where no *K. pneumoniae* was isolated.

Although stringent infection control measures were carried out, seven more ESBL-producing *K. pneumoniae* were isolated during the hospitalization of the five outbreak cases; among them, four, one, and one strains were isolated from cases 2, 3, and 5, respectively, and another one was isolated from a new case (case 7, Figure 1) in this NICU. Unfortunately, we failed to recover four of the strains (three from case 2 and one from case 5) because of poor preservation; therefore, they could not be used for subsequent experiments.

From March 27, 2015 (the discharge date of the last patients with ESBL-producing *K. pneumoniae*), over a period of six months, no further ESBL-producing *K. pneumoniae* were isolated in the NICU.

Searching for Outbreak-Related Strains Using PFGE

PFGE was used to screen outbreak-related *K. pneumoniae* strains among the strains isolated from the same hospital during or around the outbreak. A total of 22 *K. pneumoniae* strains, which were isolated between January 4 and March 17, were analyzed by PFGE. Interestingly, two

strains (CF125 and CF934) were clustered together with the eight outbreak strains (Figure 2). CF125 was isolated from case 1 on January 20, 23 days before the outbreak. CF934 was isolated from the tip of a trachea cannula of a new case (case 6, Figure 1) who had been in NICU before, on January 17, 26 days before the outbreak.

PFGE divided the ten outbreak-related strains into five different PFGE types (PT01–PT05). The dominant PFGE type (PT04) contained five isolates. The other four PFGE types showed one to three bands that were different to PT04. All ten isolates were the "same" or "closely-related" strains according to the interpretation criteria of PFGE patterns proposed by Tenover et al.[29] The other 20 *K. pneumoniae* strains isolated from the same hospital during or around the outbreak showed more than 20 bands that were different to outbreak-related strains.

Using MLST, all ten outbreak-related strains were assigned as ST471 (allele numbers of 3, 1, 1, 68, 4, 1, and 39 for *gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB,* respectively). By searching the *K. pneumoniae* MLST database (http://bigsdb.pasteur.fr.), we found that there was only one ST471 strain in the database, which was isolated from a human from Spain in 2012.
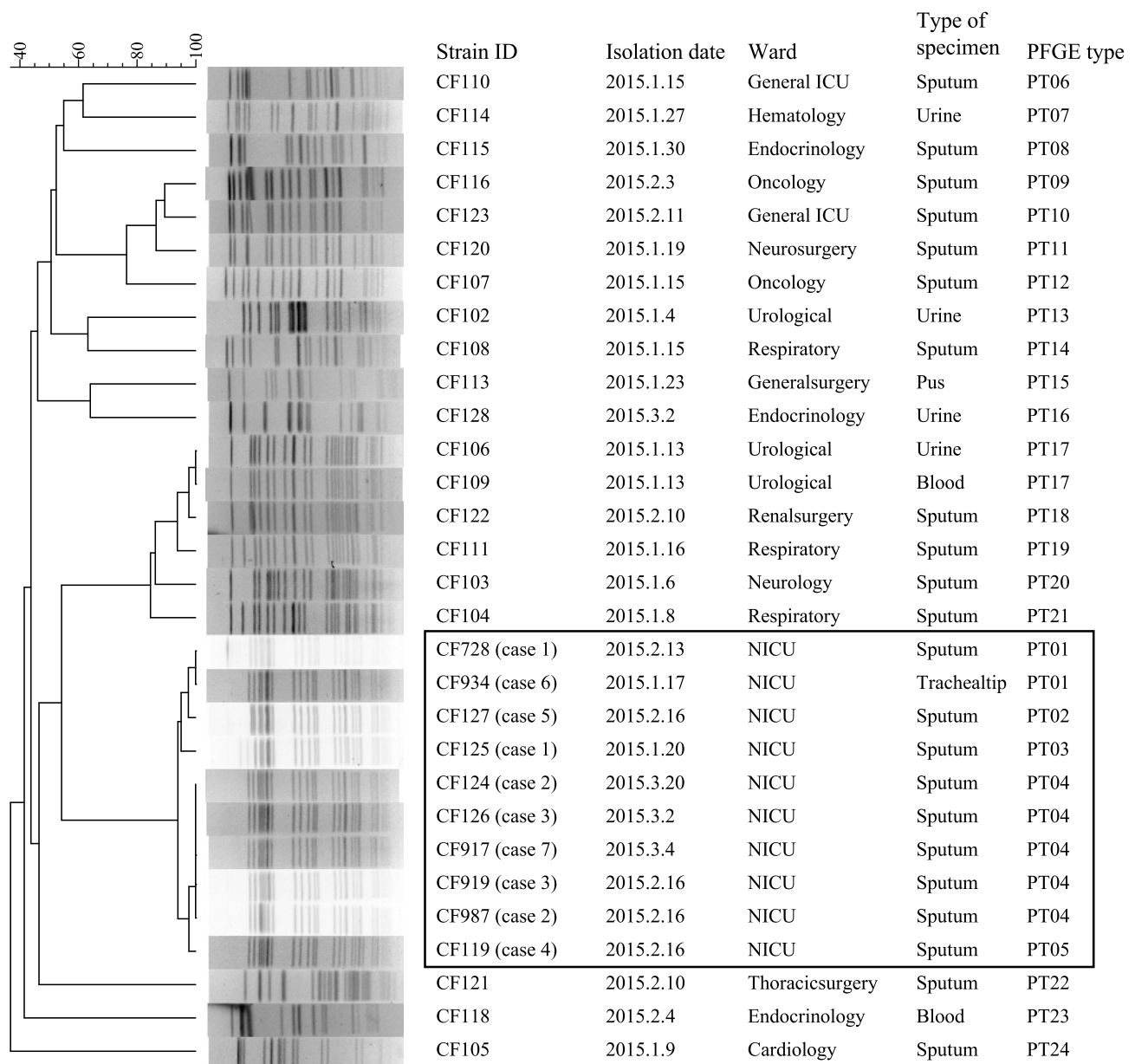
Figure 2 Clustering of the 30 *K. pneumoniae* strains based on their PFGE patterns. The outbreak and outbreak-related strains are framed. The information for strain ID, isolation date, ward, type of specimen, and PFGE type is listed to the left of the patterns.
Abbreviations: NICU, neonatal intensive care unit; PFGE, pulsed-field gel electrophoresis.

Strain ID	Isolation date	Ward	Type of specimen	PFGE type
CF110	2015.1.15	General ICU	Sputum	PT06
CF114	2015.1.27	Hematology	Urine	PT07
CF115	2015.1.30	Endocrinology	Sputum	PT08
CF116	2015.2.3	Oncology	Sputum	PT09
CF123	2015.2.11	General ICU	Sputum	PT10
CF120	2015.1.19	Neurosurgery	Sputum	PT11
CF107	2015.1.15	Oncology	Sputum	PT12
CF102	2015.1.4	Urological	Urine	PT13
CF108	2015.1.15	Respiratory	Sputum	PT14
CF113	2015.1.23	Generalsurgery	Pus	PT15
CF128	2015.3.2	Endocrinology	Urine	PT16
CF106	2015.1.13	Urological	Urine	PT17
CF109	2015.1.13	Urological	Blood	PT17
CF122	2015.2.10	Renalsurgery	Sputum	PT18
CF111	2015.1.16	Respiratory	Sputum	PT19
CF103	2015.1.6	Neurology	Sputum	PT20
CF104	2015.1.8	Respiratory	Sputum	PT21
CF728 (case 1)	2015.2.13	NICU	Sputum	PT01
CF934 (case 6)	2015.1.17	NICU	Trachealtip	PT01
CF127 (case 5)	2015.2.16	NICU	Sputum	PT02
CF125 (case 1)	2015.1.20	NICU	Sputum	PT03
CF124 (case 2)	2015.3.20	NICU	Sputum	PT04
CF126 (case 3)	2015.3.2	NICU	Sputum	PT04
CF917 (case 7)	2015.3.4	NICU	Sputum	PT04
CF919 (case 3)	2015.2.16	NICU	Sputum	PT04
CF987 (case 2)	2015.2.16	NICU	Sputum	PT04
CF119 (case 4)	2015.2.16	NICU	Sputum	PT05
CF121	2015.2.10	Thoracicsurgery	Sputum	PT22
CF118	2015.2.4	Endocrinology	Blood	PT23
CF105	2015.1.9	Cardiology	Sputum	PT24

Antibiotic Susceptibility and Characterization of Resistance Genes

All 10 ESBL-producing *K. pneumoniae* strains showed same results for the antibiotic susceptibility test. All strains showed insensitivity to ampicillin, ampicillin/sulbactam, amoxicillin/clavulanate, cefazolin, cefotaxime, cefepime, trimethoprim-sulfamethoxazole, and aztreonam, and were susceptible to other 14 antibiotics (cefoxitin, ceftazidime, imipenem, meropenem, amikacin, kanamycin, azithromycin, tetracycline, doxycycline, tigecycline, ciprofloxacin, levofloxacin, chloramphenicol, and colistin). All ten *K. pneumoniae* strains were confirmed as positive for ESBL production using double-disk diffusion tests.

By PCR and sequence alignment of beta-lactamase genes, all 10 isolates were shown to harbor $bla_{CTX-M-15}$, bla_{TEM-1}, $bla_{SHV-108}$, and bla_{OXA-1} genes, and tested negative for bla_{PER}, and bla_{VEB}. Based on the WGS results, we further characterized the antibiotic resistance genes among the 10 isolates. In addition to the four beta-lactamase genes ($bla_{CTX-M-15}$, bla_{TEM-1}, $bla_{SHV-108}$, and bla_{OXA-1}),

all isolates were confirmed to possess genes for resistance to aminoglycoside (*aac(3)-II, aac(6')-Ib, aph(33)-Ib* and *aph(6)- Id*), sulfonamide (*sul2*), fosfomycin (*fos*A), trimethoprim (*dfrA14, dfrA22*), chloramphenicol (*catB3*), polymyxin (*arnA*), and bacitracin (*bacA*). Furthermore, several resistance pumps were identified, including a plasmid-mediated tetracycline resistance pump (*tet_efflux*).

Comparison of Outbreak Isolates Based on WGS-Based SNPs

WGS and MCG typing were performed for the 10 ESBL-producing *K. pneumoniae* strains, with the aim of using the SNPs found in their genomes to determine a putative transmission map of this outbreak. Genomic comparisons revealed a total of 7 MCG SNPs among the 10 strains (Figure 3). The evolutionary relationships based on MCG typing of the 10 strains are outlined and presented in Figure 4. The strains isolated from case 6 (CF934) in January showed the same SNP profile as the reference strain *K. pneumoniae* KPR0928. The first strain isolated from case 1 (CF125) had one SNP (T608763C) that was different to the reference strain. Another case 1 strain (CF728) had one more

Figure 3 Heat map of SNP distribution of ten ESBL-producing *K. pneumoniae*.

SNP (C154003T). The first strain (CF987) isolated from case 2 had the same SNP (T608763C) to that of the first case 1 strain (CF125). Another case 2 strain (CF124) had one more SNP (G953229A). The strains isolated from cases 4 (CF119) and 5 (CF127), both of which were isolated on February 16, had one (G2665757T) and two (C996839T, C2820669A) SNPs that were different to the case 1 and 2 strains (CF125, CF987), respectively. The first strain (CF919) of case 3, which was also isolated on February 16, had one SNP (G2550784A) that was different to the case 4 strain (CF119). The second case 3 strain (CF126), which was isolated 14 days later, showed one more SNP (G3771456A).

Identification of the Origin and the Construction of a Putative Transmission Route of the Outbreak

The genomic and epidemiological information were integrated to construct the transmission route. The most likely transmission route is shown in Figure 5.

First, case 6 was suspected of being the source of the outbreak isolates, because the historical strain from case 6 showed the same SNP patterns to the reference strain *K. pneumoniae* KPR0928. This was also supported by the epidemiological information. The case 6 strain was the earliest isolated ESBL-producing *K. pneumoniae* strain, which was isolated 1 month before the February 16 outbreak. Thus, case 6 was the most likely source of the outbreak isolates.

Second, the first strains isolated from cases 1 and 2 showed one SNP difference to the isolates of case 6, suggesting that the strains from cases 1 and 2 were not transmitted from case 6. However, combining the epidemiological information, that the first strain of case 1 was isolated on January 20, closer to the isolation of case 6 strain; and the first strain of case 2 was isolated on February 16, suggesting that the most possible transmission route was that this ESBL-producing *K. pneumoniae* strain was passed from case 6 to case 1 and then to case 2. Interestingly, the second strains from both cases 1 and 2 had one SNP difference with the first strains, suggesting that there were mutations in vivo.

Third, the strains from cases 4 and 5 had one and two SNPs different to the first strains of cases 1 and 2. However, the second strain of case 1 was isolated three days before the isolation of the strains from cases 4 and 5 and showed another SNP, which suggested that the strains of cases 4 and 5 were not transmitted from case 1. Thus, case 2 was the most likely source of the strains isolated from cases 4 and 5.
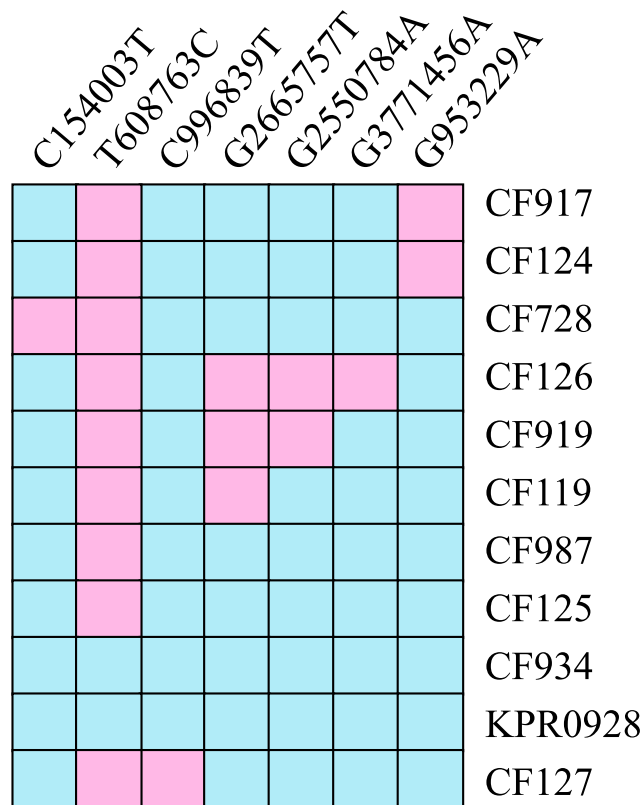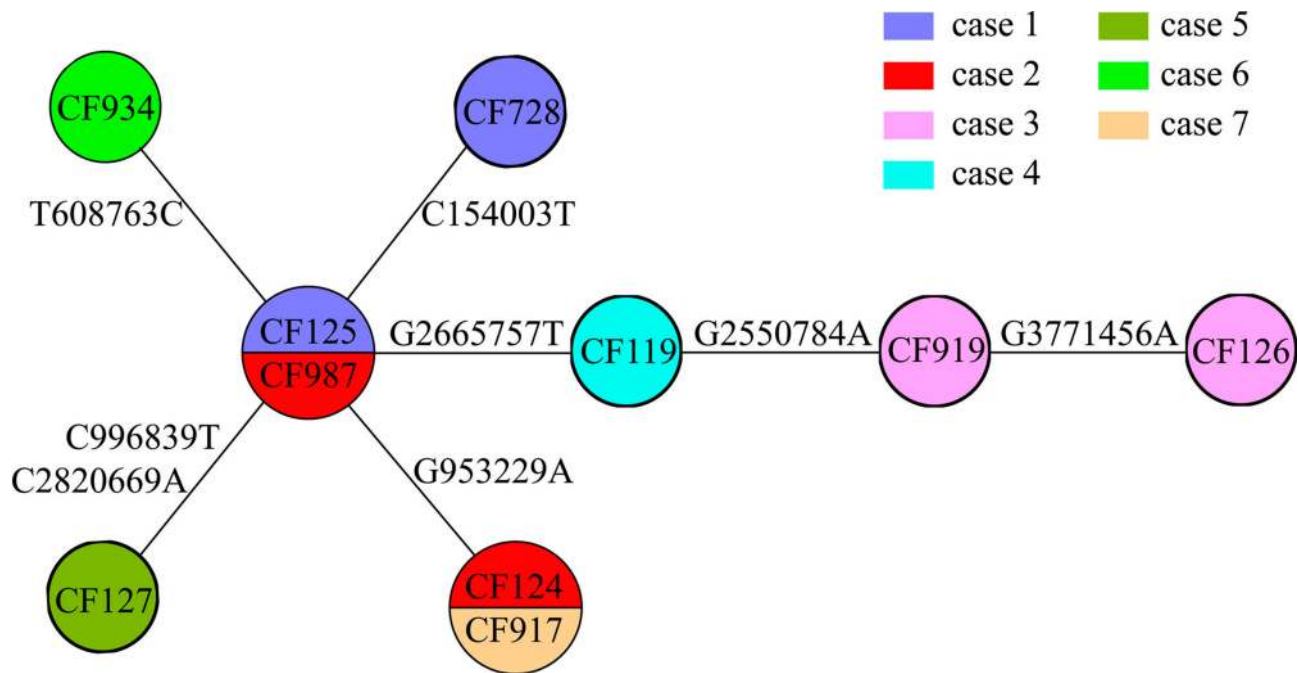
Figure 4 Evolutionary relationships based on MCG typing of ten ESBL-producing *K. pneumoniae*. Isolates from different cases are indicated in different colors. SNPs are marked on the connecting lines.

Fourth, the first strain of case 3, which was also isolated on February 16, had one SNP difference with the case 4 strain. The second case 3 strain, which was isolated 15 days later, showed one more SNP. Thus, the isolates of case 3 were transmitted from case 4, which then persisted in case 3 and mutated in vivo.

Fifth, the strain from case 7 was isolated 20 days after the February 16 outbreak and showed same SNP profile as the second strain of case 2, suggesting that case 2 directly transmitted this strain to case 7.

On the whole, consistent with the genomic and epidemiological information, the integrated map identified that: (1) Case 6 was the source of the February 16 outbreak; (2) before the outbreak, the ESBL-producing *K. pneumoniae* was a long-term resident in case 1; (3) case 2 played a key role, as a "superspreader", in the outbreak, probably directly causing cases 4, 5, and 7, and indirectly causing case 3.

Discussion

In this study, we described an outbreak caused by ESBL-producing *K. pneumoniae* in a NICU of a large university hospital in China. The ESBL-producing *K. pneumoniae* isolates belonged to ST471, and all harbored $bla_{CTX-M-15}$, bla_{TEM-1}, $bla_{SHV-108}$, and bla_{OXA-1} beta-lactamase genes. ST471 is a highly uncommon ST, which has only been

reported in one human case in Spain and clinical bovine mastitis in Tunisia.[30] The Tunisian study pointed out the necessity to improve farm management to avoid the dissemination in this sector of ESBL-producing *K. pneumoniae*. It is worth noting that the outbreak reported here occurred in Chifeng City, which belongs to Inner Mongolia, the largest pastoral area in China. Therefore, a future investigation should determine whether this ST471 strain infected humans via cattle, sheep, or other livestock. Furthermore, as far as we know, this is not only the first study to report an outbreak caused by *K. pneumoniae* belonging ST471, but also is the first to report an outbreak caused by a *K. pneumoniae* strain co-harboring the $bla_{CTX-M-15}$, bla_{TEM-1}, $bla_{SHV-108}$ and bla_{OXA-1} genes, which suggests that more attention should be paid to the *K. pneumoniae* isolates producing epidemic ESBLs.

CTX-M-15 is the main CTX-M-type of clinical ESBL-producing *K. pneumoniae* in China and other parts of the world.[31–37] Furthermore, $bla_{CTX-M-15}$-positive *K. pneumoniae* has been also widely detected in various animals, including farm cattle, pet dogs, and wild birds,[38–40] indicating communication between the genetic pools of the $bla_{CTX-M-15}$ gene in animals and humans. In future studies, we should investigate how these ESBLs are transmitted from animals to humans, and then implement measures to block this route to avoid such infections and outbreaks. TEM-1 is the main TEM-type of
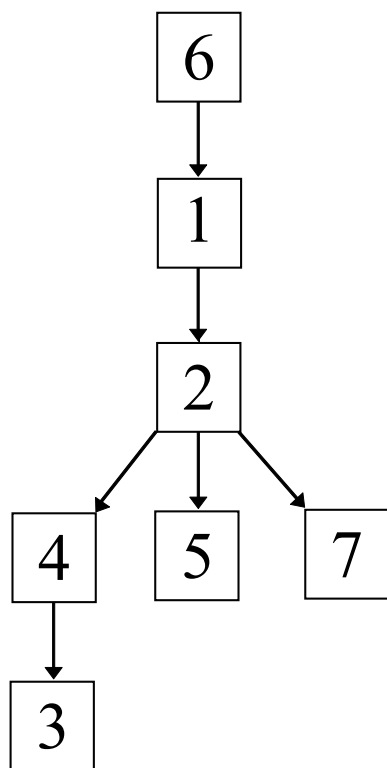
Figure 5 Putative map of ESBL-producing *K. pneumoniae* transmission during the outbreak. The transmission map was constructed using genetic and patient trace data. Nodes represent patients, and arrows indicate a transmission event directly from one patient to another.

K. pneumoniae isolated from Tanzania, India, and Iran.[41–43] OXA-1 and SHV-108 are rarely detected from *K. pneumoniae*. In a recently multicenter survey of ESBL-producing *K. pneumoniae* causing intra-abdominal infections from nine tertiary hospitals in China, no TEM-type and OXA-1 strain was detected, and the most common genotype for *K. pneumoniae* was SHV-2a.[32] However, in another survey of *K. pneumoniae* from pediatric patients in Shanghai, China, TEM-1 and OXA-1 were detected in 68.3% and 14.6% of strains.[44] Although there is no global survey of the distribution of these resistance genes at present, it can be seen from the current research reports that there are regional differences in the distribution of these beta-lactamase genes between different countries, different cities, even between different hospitals of the same region.

PFGE used to be the gold standard for molecular subtyping of outbreaks. However, although PFGE could identify outbreak-related strains, it could not reveal the evolutionary relationship and transmission route between them. Our previous research showed that WGS and MCG typing could reveal the details of transmission within a *K. pneumoniae* nosocomial outbreak.[16] MCG typing

was first used to study the population structure of *Streptococcus suis* and then *Legionella pneumophila*.[17,26] In the present study, we further confirmed that WGS and MCG typing can reveal the fine transmission map and identify the "superspreader" of an outbreak. If WGS and MCG typing were applied in the early stage of an outbreak, the outbreak could be controlled. In addition, this method has the potential to be used to track other human-borne pathogens, such as *Neisseria meningitidis, Bordetella pertussis*, and *Streptococcus pneumoniae*.

Conclusion

In summary, we describe the integration of epidemiological information with high-resolution WGS and MCG typing to enhance the investigation of an outbreak of ESBL-producing *K. pneumoniae* infection. Our data showed that epidemiological inferences are greatly improved by interpretation in the context of WGS and that *K. pneumoniae* strains isolated from the same outbreak contain sufficient SNP differences to refine the epidemiological linkages on the basis of genetic lineage.

Ethics and Consent Statement

This study was approved by the scientific and ethics committees of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. All clinical specimens from patients were collected for diagnostic testing in hospitals at the request of the attending doctors. All experiments on *K. pneumoniae* strains were reviewed and approved by the scientific and ethics committees. All experiments were performed in accordance with relevant guidelines and regulations. Furthermore, to protect patient privacy, the hospital set up a patient privacy and medical record management system according to the criminal procedure law, civil procedure law, tort liability law, and medical malpractice law in China. Excepting for the patient's doctor, no one can enter the management system. Although no written informed consent was provided by patients or their guardians, the above measures were considered sufficient to protect the patients' privacy.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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