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An outbreak of infections caused by extensively drug-resistant *Klebsiella pneumoniae* strains during a short period of time in a Chinese teaching hospital: epidemiology study and molecular characteristics

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ABSTRACT

In this study, we comprehensively described the clinical risk factors, outcome, epidemiology, and molecular basis associated with an outbreak of extensively drug-resistant KPC-2–producing *Klebsiella pneumoniae* involving 15 patients in a teaching hospital from May 1 to June 27, 2013. Most of the patients were elderly and received long-term hospital treatment, and 40.0% (6/15) of them were dead. All strains carried bla_{KPC-2}, *rmtB*, $bla_{CTX-M-65}$, bla_{SHV-11}, *oqxA*, *oqxB*, and *aac(6′)-Ib-cr* and even harbored additional other resistance genes, such as *armA*, $bla_{CTX-M-1}$, bla_{TEM-1}. bla_{KPC-2}, *rmtB*, and $bla_{CTX-M-65}$ were located on the same ~54.2-kb plasmid, and conjugation experiments further proved the cotransferable characteristic. Alterations of outer membrane proteins were confirmed by sodium dodecyl sulfate - polyacrylamide gelelectrophoresis and sequencing, which can lead to a drastic change in the permeability of cells. All isolates belonged to the clone complex 258, spreading rapidly across the world. Our study demonstrated that a high degree of awareness and surveillance of those drug resistance determinants is urgently needed.

1. Introduction

Klebsiella pneumoniae is an opportunistic pathogen that is highly adapted to the hospital environment, which is associated with various nosocomial infections, such as pneumonia, bloodstream infections, and urinary tract infections. *K. pneumoniae* is second only to *Escherichia coli* as a cause of nosocomial gram-negative infections (Tantry and Rahiman, 2012). The number of drug-resistant strains has been increasing dramatically following the widespread and at times indiscriminate use of antibiotics in clinics, often leading to clinical failure, prolonged hospitalization, increased morbidity, mortality, and increased health care costs (Barriere, 1992).

Carbapenems possess a broad spectrum of activity and remain an important therapeutic option against gram-negative strains. According to CHINET, a leading antimicrobial resistance surveillance networks in China, carbapenem-resistant *K. pneumoniae* escalated from 0.7% in 2006 to 10% in 2013 (Hu et al., 2014), which has mainly resulted from the rapid dissemination of KPC-producing *K. pneumoniae*, and most studies focused on multidrug-resistant (MDR) strains (Li et al., 2012b). Infections caused by extensively drug-resistant (XDR), as defined by Magiorakos et al. (2012), have challenged the clinically available therapeutic options. In the present report, we comprehensively described an outbreak of infections caused by XDR *K. pneumoniae* strains during a short period of time from May to July 2013.

2. Materials and methods

2.1. Bacterial strains

According to the standardized international antimicrobial susceptibility profile with which to describe acquired resistance profiles in Enterobacteriaceae, XDR was defined as nonsusceptibility to at least 1 agent in all but 2 or fewer antimicrobial categories (Magiorakos et al., 2012). Fifteen XDR *K. pneumoniae* were selected for research from 587 consecutive nonduplicate *K. pneumoniae* clinical isolates collected at the First Affiliated Hospital of Wenzhou Medical University between January 2013 and August 2013. Initially, bacterial and antimicrobial susceptibilities were conducted by a VITEK 60 system (bioMérieux, Lyons, France). Clinical records of patients were examined retrospectively.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by agar dilution and interpreted according to CLSI (2014), except for tigecycline and colistin, which were tested by broth microdilution using Mueller–Hinton broth and interpreted according to the recommendation of the

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European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). *E. coli* ATCC 25922 was used for quality control.

2.3. Investigation of resistance determinants

Total DNAs of all isolates were obtained with an AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Scientific, Union City, CA, USA). Resistance genes were amplified by PCR and sequenced. All isolates were screened for the presence of 3 kinds of resistance-determining factors: i) the β-lactamase–encoding genes (Jacoby, 2009; Shahcheraghi et al., 2009): $bla_{\text{CTX-M-group 1}}$, $bla_{\text{CTX-M-group 9}}$, bla_{TEM}, bla_{SHV}, bla_{BES} (coding for ESBLs); bla_{CMY}, bla_{MOX}, bla_{FOX}, bla_{LAT} (coding for AmpC); and bla_{KPC}, bla_{GES}, bla_{SPM}, bla_{IMP}, bla_{VIM}, bla_{NDM}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-48}}$ (coding for carbapenemases); ii) plasmid-mediated quinolone resistance (PMQR) genes (Li et al., 2012c): *qepA*, *aac(6′)-Ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oqxA*, *oqxB*; iii) 16S rRNA methylase genes (Yu et al., 2009): *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, and *npmA*. Nucleotide sequences were analyzed and compared by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Plasmid analysis and Southern hybridization

In order to distinguish between the chromosomal or plasmid location of the main resistance genes, plasmids were extracted by the Qiagen Plasmids Midi Kit and separated by electrophoresis. Then the DNA in the gel was transferred to a positively charged nylon membrane (Roche Diagnostics, Branford, CT, USA) by the capillary method and were hybridized with the labeled bla_{KPC}, *rmtB*, and $bla_{\text{CTX-M-group 9}}$ probes according to the manufacturer's instructions Detection Starter Kit II (Roche, Sant Cugat del Vallès, Spain). The plasmids of *E. coli* V517 (2.1, 2.7, 3.0, 3.9, 5.2, 5.6, and 54.2 kb) served as size marker.

2.5. Conjugation experiments

The potential for conjugational transfer of resistance genes was examined by biparental matings using an azide-resistant *E. coli* J53 as the recipient strain, and transconjugants were selected on Mueller–Hinton agar plates containing 100 μg/mL sodium azide for counterselection and 100 μg/mL ampicillin to select for plasmid-encoded resistance (Li et al., 2012a). MICs of several representative agents for transconjugants were tested and analyzed by Southern hybridization like donors above to confirm the mobilizable resistance genes.

2.6. Examination of outer membrane proteins and porin genes

Cultures were grown in both Luria Bertani broth (high-osmolarity medium) and Nutrient Broth broth (low-osmolarity medium), as the expression of OmpK35 was down-regulated in a high-osmolarity culture medium (Domenech-Sanchez et al., 2003). Outer membrane proteins (OMPs) were obtained by ultrasonic crushing method and analyzed via SDS-PAGE as previously described protocol (Shi et al., 2013), using *K. pneumoniae* ATCC 13883 (an isolate with known expression of both porins) as a control for porin profiles. The structural genes of OmpK35 and OmpK36 were also amplified specifically, and the predicted amino acid sequences were compared with reference protein sequences of NTUH-K2044 and *K. pneumoniae* C3 (Zhang et al., 2014).

2.7. Bacterial clonal relatedness

Bacterial clonal relatedness was established by pulsed-field gel electrophoresis (PFGE) using the *XbaI* restriction enzyme (Takara Bio, Dalian, China), and the result was analyzed and interpreted according to the initial criteria (Hu et al., 2013). Multilocus sequence typing (MLST) was also performed to determine phylogenetic relationships according to protocols available at an MLST Web site (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html).

3. Results and discussion

3.1. Patient data and clinical characteristics

All the strains were isolated from sputum, except for one that was cultured from catheter, between May 1 and June 27 (Table 1). Patients were mainly hospitalized in the neurosurgery ward, which was likely to be the origin of this outbreak. The medical records showed that the patient infected by FK601 and FK720 was transferred to neurosurgery and intensive care unit (ICU), respectively, on April 29 and June 5; FK625 was detected in neurosurgery following FK601. This analysis highlights the importance of microbiological screening for XDR strains among patients sharing the same ward with a known XDR carrier.

The retrospective clinical data showed that there were some common characteristics among the patients, such as senility and long-term hospitalization, which were the main risk factors of nosocomial infection identified in a previous study (Woodford et al., 2004). The patients were primarily elderly (median age 60 years, range 50–69 years) who coexisted similar underlying diseases and had been submitted to multiple invasive procedures, such as tracheostomy. The median number of days of hospitalization from admission to identification of XDR *K. pneumoniae* was 19 days (range 7–51 days). Previous studies reported a 2- to 4-fold increase in the *K. pneumoniae* colonization rates after 2 weeks of hospitalization, which were overall hospitals rates and were not associated with certain awards (Podschun and Ullmann, 1998). Among evaluated patients, 40.0% (6/15) died due to the infection caused by the XDR *K. pneumoniae*. The remaining patients (60.0%; 9/15) improved and were discharged. Isolates demonstrating an XDR phenotype are frequently responsible for ventilator-associated pneumonia and a major cause of death, morbidity, and costs in ICUs (Rouze and Nseir, 2013), which probably associated with the mortality in the present study. The isolates were broadly resistant to many antibiotic classes, and the infections caused by these *K. pneumoniae* were proved extremely difficult to eradicate. Physical isolation of infected patients was the crucial first step adopted to confine the outbreak. The importance of an aseptic technique was reemphasized to all health care workers, including repeated practices of sterilization of the instruments used in the department, the surfaces (at least twice a day), and the environment. In particular, the importance of hand hygiene was highlighted, as well as the change of gloves, gowns, and other personal protective equipment used during the care of patients. The outbreak was eventually controlled and eliminated. There had been no more cases of KPC-2–producing isolates since July 16, 2013.

3.2. Antimicrobial susceptibility testing

The MICs of a variety of antimicrobial agents tested against the strains were shown in Table 2. Fifteen isolates displayed resistance to all categories of β-lactam antimicrobials, β-lactam/inhibitor combinations, aminoglycosides, quinolones, and other clinical antibacterial agents except for tigecycline and colistin, which were defined as XDR bacteria following the guidelines described by a group of international experts (Magiorakos et al., 2012).

3.3. Antimicrobial resistance determinants and Southern hybridization

After sequencing the positive PCR products, the $bla_{\text{CTX-M-group 9}}$ ($bla_{\text{CTX-M-65}}$), $bla_{\text{SHV-1}}$ ($bla_{\text{SHV-11}}$), *aac(6′)-Ib-cr*, *oqxA*, *oqxB*, $bla_{\text{KPC-2}}$ ($bla_{\text{KPC-2}}$), and *rmtB* genes were identified in all isolates, even several strains harbored additional resistance genes, such as *armA*, $bla_{\text{CTX-M-1}}$, and $bla_{\text{TEM-1}}$. That is to say each strain carried at least 3 different kinds of resistance determinants, which can specifically hydrolyze β-lactams, aminoglycosides, and quinolones, representing the first-line agents in clinical anti-infective target therapy. Generally, KPC-2–producing *K. pneumoniae* has the MDR feature, and even more serious is the rapid global dissemination of KPC genes. Horizontal transfer of KPC resistance