Analysis of Drug Resistance Determinants in *Klebsiella pneumoniae* Isolates from a Tertiary-Care Hospital in Beijing, China

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**Abstract**

**Background:** The rates of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) isolates among *Enterobacteriaceae* isolates, particularly *Klebsiella pneumoniae*, have risen substantially worldwide.

**Methodology/Principal Findings:** To better understand the molecular mechanisms of drug resistance in *K. pneumoniae*, we analyzed the drug resistance determinants for *K. pneumoniae* isolates collected from the 306 Hospital, a tertiary-care hospital in Beijing, China, for the period of September 1, 2010-October 31, 2011. Drug susceptibility testing, PCR amplification and sequencing of the drug resistance determinants were performed. Conjugation experiments were conducted to examine the natural ability of drug resistance to disseminate among *Enterobacteriaceae* strains using a sodium azide-resistant *Escherichia coli* J53 strain as a recipient. Among the 223 consecutive non-repetitive *K. pneumoniae* isolates included in this study, 101 (45.3%) were extended-spectrum beta-lactamases (ESBLs) positive. The rates of MDR, XDR, and PDR isolates were 61.4% (n = 137), 22.0% (n = 49), and 1.8% (n = 4), respectively. Among the tested drug resistance-associated genes, the following ones were detected at relatively high rates *blaCTX-M-10* (80, 35.9%), *aacC2* (73, 32.7%), *dhfr* (62, 27.8%), *qnrS* (58, 26.0%), *aadA4* (57, 25.6%), *aadA1* (56, 25.1%). Results from conjugation experiments indicate that many of the drug resistance genes were transmissible.

**Conclusions/Significance:** Our data give a “snapshot” of the complex genetic background responsible for drug resistance in *K. pneumoniae* in China and demonstrate that a high degree of awareness and monitoring of those drug resistance determinants are urgently needed in order to better control the emergence and transmission of drug-resistant *K. pneumoniae* isolates in hospital settings.

**Citation:** Li B, Yi Y, Wang Q, Woo PCY, Tan L, et al. (2012) Analysis of Drug Resistance Determinants in *Klebsiella pneumoniae* Isolates from a Tertiary-Care Hospital in Beijing, China. PLoS ONE 7(7): e42280. doi:10.1371/journal.pone.0042280

**Editor:** Igor Mokrousov, St. Petersburg Pasteur Institute, Russian Federation

**Received** April 17, 2012; **Accepted** July 2, 2012; **Published** July 31, 2012

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**Funding:** This work was supported by the National Basic Research Program of China (2012CB518700), the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-J-6), the National Natural Science Foundation of China (grant No. 30700975), and the Merieux Research Grant program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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**Introduction**

The emergence and rapid spread of drug-resistant *Klebsiella pneumoniae* isolates is becoming a serious antibiotic management problem and causing a great concern worldwide [1–6]. For example, by late 2009, the number of unique protein sequences for beta-lactamases exceeded 890 (http://www.lahey.org/Studies) [7]. There is an increasing recognition of isolates producing newer beta-lactamases including the extended-spectrum beta-lactamase (ESBL), carbapenem-hydrolyzing enzymes (e.g., *K. pneumoniae* carbapenemase [KPC] types and the metallo-beta-lactamases [MBLs]) [8–18]. Since the production of newer beta-lactamases is frequently accompanied by broad-spectrum resistance, the ESBL positivity together with the existence of newer beta-lactamases should be monitored closely as the emergence of those highly drug-resistant *K. pneumoniae* strains will pose a serious impact on the remaining therapeutic options [19–22]. In a study based on the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database, the rate of ESBL production was highest among the *K. pneumoniae* isolates collected in Latin America, followed by Asia/Pacific Rim, Europe, and North America (44.0%, 22.4%, 13.3% and 7.5%, respectively) [23]. Thus the potential of drug resistant *K. pneumoniae* to be a global health problem is great and more intensive surveillance and more in-depth investigation into the molecular mechanisms of drug resistance in *K. pneumoniae* isolates are necessary in order to provide information for the development of effective molecular diagnostic methods and novel drugs against *K. pneumoniae* infection.

In the face of increasing resistance among multidrug-resistant (MDR) gram-negative organisms for which no adequate therapeutic options exist, a joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centers for...
Disease Control and Prevention (CDC) recently created a standardized international definitions for MDR, extensively drug-resistant (XDR) and pandrug-resistant (PDR) with an aim to enhance the comparability of data and promote better comprehension of the problem of highly drug-resistant bacteria [24]. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories [24]. Though there are already many reports of drug-resistant K. pneumoniae worldwide, the extent of MDR, XDR and PDR K. pneumoniae isolates among patients is largely unknown. We thus in this study sought to determine the prevalence of MDR, XDR and PDR strains and to analyze the drug resistance determinants for K. pneumoniae isolates collected from patients being treated in the 306 Hospital, a tertiary-care hospital in Beijing, China, for the period of September 1, 2010-October 31, 2011 with an aim to better understand the current situation as well as the genetic background of the drug resistant K. pneumoniae isolates from hospital settings.

Methods

Ethics Statement

All of the investigation protocols in this study were approved by the institutional ethics committee of the 306 Hospital, Beijing, China. Written consent was given by the patients for their information to be stored in the hospital database and used for research. Permission for using the information in the medical records of the patients for research purposes was obtained from the 306 Hospital. The Institute ethics committee of the 306 Hospital reviewed that relevant ethical issues in this study were well considered.

Study Population, Bacterial Isolate Identification, and Drug Susceptibility Testing

This is a prospective surveillance study. Consecutive K. pneumoniae isolates were collected from unique patients being treated in the 306 Hospital in Beijing, China (which is a 1,100-bed tertiary-care hospital serving approximately 25,000 in-patients per year) for the period of September 1, 2010-October 31, 2011. In the case of duplicate patient samples, the first collected isolate was chosen. All strains were cultured in Luria–Bertani (LB) medium. The K. pneumoniae strains were confirmed by phenotypic tests and 16S rDNA sequencing. Drug susceptibility testing (DST) for the K. pneumoniae strains was performed using the bioMérieux VITEK-2 AST-GN13 system following manufacturer’s instructions. The following 18 drugs were tested: ampicillin (AMP), piperacillin/tazobactam (TZP), ampicillin/sulbactam (SAM), cefazolin (CFTZ), ceftriaxone (CRO), cefazidime (CAZ), cefepime (FEP), ceftazidime (CTT), ertapenem (ETP), imipenem (IMP), aztreonam (ATM), ceftriaxone (CIP), levofloxacin (LVX), gentamicin (GM), tobramycin (TOB), amikacin (AMK), trimethoprim-sulfamethoxazole (SXT), furadantin (FD). The ESBLs were detected by the bioMérieux VITEK-2 AST-GN13 test (which is claimed to be a confirmatory ESBL test). In some cases, the ESBL positivity was further confirmed by the double disk diffusion method [25]. Escherichia coli strains ATCC 25922 and ATCC 35218, K. pneumoniae strain ATCC 700605 and Pseudomonas aeruginosa strain ATCC 27853 were used as quality control strains for the DST. Clinical records of patients from whom the K. pneumoniae isolates were obtained were reviewed retrospectively.

PCR Amplification and Sequencing

Genomic DNA was extracted using DNeasy Tissue kit (Qiaegen; Valencia, CA, USA). Drug resistance-associated genes were detected by PCR and sequencing using 37 pairs of primers listed in Table 1. Direct sequencing of positive amplicons was conducted. The primers were synthesized by the Beijing Genomics Institute (BGI, China). PCR was performed in a 30-μL reaction mixture consisting of 5 μL of 10×PCR buffer, 2.5 units of Taq DNA polymerase (Takara), 0.2 mM of dNTPs, 0.4 μM each of the primer, and 1 μL chromosomal DNA. All reaction mixtures were subjected to 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. PCR products were purified and sequenced bi-directionally with the same primers used for PCR by the Beijing Genomics Institute (BGI, China). DNA sequences were annotated using the BLAST program at http://www.ncbi.nlm.nih.gov. Mutations in the gyrA and parC genes were identified by comparing the DNA sequences with gyrA and parC sequences of the K. pneumoniae (GenBank accession numbers DQ673325 and NC009648 for gyrA and parC, respectively).

Conjugation Experiments, Plasmid Analysis, and MLST Analysis

Transfer of resistance genes by conjugation experiments were carried out in LB broth using clinical isolates as donors and the E. coli J53AzAR as the recipient as described previously [26]. Cultures of donor and recipient cells in logarithmic phase (0.5 mL each) were added to 4 mL of fresh LB broth and incubated overnight without shaking. Transconjugants were selected on LB plates containing 100 μg/mL sodium azide for counterselection and 100 μg/mL ampicillin to select for plasmid-encoded resistance. The drugs tested were purchased from Sigma Chemical Co. Plasmid DNA from the K. pneumoniae donor strains and E. coli transconjugants were prepared using the Plasmid Maxprep Kit (Vigorous Biotechnology, Beijing, China) and were separated on 0.7% agarose gels. Genotyping was determined by MLST analysis. MLST with seven genes (gyrA, infB, mdh, pgi, phoE, tpsB and tonB) was performed on isolates according to the protocol described on the K. pneumoniae MLST website (www.pasteur.fr/mlst). Sequence types (STs) were assigned by using the MLST database (www.pasteur.fr/mlst/Kpneumoniae.html).

Statistical Analysis

SPSS software (version 15.0) was used for data analysis. Categorical variables were compared with the chi-square test or Fisher’s exact test. A p value of <0.05 was considered to be statistically significant.

Results

Demographic and Clinical Characteristics of the Patients

From September 1, 2010 to October 31, 2011, a total of 223 non-repetitive patients at the 306 Hospital who had K. pneumoniae isolates were subjected to DST using 18 antibiotics. Among which, 137 (61.4%) were MDR isolates, 49 (22.0%) were XDR isolates, 4 (1.8%) were PDR isolates, and 33 (14.8%) were other types of isolates. The proportion of the male and female were 73.5% (n = 164) and 26.5% (n = 59), respectively. Sixty-eight (30.5%) of the patients were Beijing residents and the rest were from other provinces of China (non-Beijing residents). The median (±SD) age of the patients was 74.0±20.3 years (range 1.0–98.0 years). The majority of the patients were from medical ward (97, 43.5%) and intensive care unit (75, 33.6%). The main source of the specimens was sputum (168, 75.3%). The proportion of the ESBL positive cases was...
The proportion of XDR (42, 41.6%) and PDR (4, 4.0%) cases was significantly higher among patients whose isolates were ESBL positive as compared with those whose isolates were ESBL negative. In addition, the proportion of MDR cases (83, 68.0%) and other types of cases (32, 26.2%) was significantly higher in patients with ESBL-negative isolates than that observed for XDR (7, 5.7%) and PDR cases (0). The detailed information on relevant demographic and clinical characteristics of the study population is summarized in Table 2.

Drug Susceptibility Patterns of the *K. pneumoniae* Isolates

DST was conducted for 223 *K. pneumoniae* isolates and the detailed information on resistance rates to all tested drugs are listed in Table 3. The highest resistance rate was observed for AMP, reaching 99.6% (n = 222), followed by resistance to FD (190, 85.2%), CTT (153, 68.6%), SXT (118, 52.9%), SAM (115, 51.6%), CFZ (114, 51.1%), CRO (110, 49.3%), CAZ (110, 49.3%), ATM (109, 48.9%), and TOB (109, 48.9%). The two carbapenems tested including ETP and IMP exhibited relatively lower resistance rates (7.2% and 5.8%, respectively). Notably, the

| Target genes | Primer sequence (5' to 3') | Amplicon size (bp) | Source of reference |
|--------------|-----------------------------|--------------------|--------------------|
| blaCTX-M-1    | GGT TAA AAA ACT CTG GCC TC  | 876 [2]            |                    |
| blaCTX-M-2    | ATG ACT CAG AGC ATT CG      | 876 [29]           |                    |
| blaCTX-M-3    | GGT GTT GTT ATC TCG TAT CCT CC | 934 [10]    |                    |
| blaCTX-M-8    | ATG AGA CAT CGC GTT AAG    | 864 [61]           |                    |
| blaCTX-M-9    | GTG ACA AAG GTG CTA CGG    | 850 [31]           |                    |
| blaCTX-M-10   | GCA GCA CCA GTA AAG TGA TG | 873 [30]           |                    |
| blaCTX-M-14   | ACA ATG ACG CTG GCA AAG TG | 512 [64]           |                    |
| blaCTX-M-25   | CAC ACG AAT TGA ATG TTC TT | 924 [61]           |                    |
| blaTEM        | KAC AAT AAT CTT GRT AAA TGC | 899 [32]           |                    |
| blaPER        | ATG ATG ACT TCG TAT CGC GT | 882 [8]            |                    |
| blaVIM        | GGT CTC ATT GTC GTG GAT GAG | 271 [27]           |                    |
| blaOXA        | TTG GTG CCA ATG ATT ACC GA | 743 [47]           |                    |
| blaCTX-M-8    | TGG CCA GAA CTG ACA GGC AAA | 462 [65]           |                    |
| blaCTX-M-9    | AAC TTT CAC AGG TGT GGG T  | 405 [65]           |                    |
| blaCTX-M-10   | AAC ATG GGG TAT CAG GGA GAT G | 190 [65]   |                    |
| dfr           | GAC AAT CTT GGT ATT GGC AA  | 357 [15]           |                    |
| qnrA          | ATT TCT CAC GCC AGG ATT TG  | 627 [62]           |                    |
| qnrB          | GAT GTG GAA AAG CAG AAA GG  | 469 [62]           |                    |
| qnrC          | GGG TTT TGG ATT TAT TGA ACG | 307 [34]           |                    |
| qnrD          | CGA GAT CAA TTTA CGG GGA ATA | 533 [33]   |                    |
| qnrS          | ACG ACA TAC TGT ACG TGC AA | 417 [28]           |                    |
| qacA4         | TGT CCA TCG TGT ATG GGC TA  | 482 [35]           |                    |
| qepA          | AAC TGC TGT ACC CCG AGG ATG TAG AT | 596 [34] |                    |
| gyrA          | GAT CGT GAA AAG CAG AAA GG  | 469 [62]           |                    |
| parC          | TAC GTC ATC ATG AGC AGG     | 460 [37]           |                    |
| aacA4         | ATG ACT GAG CAT GAC TGG     | 540 [38]           |                    |
| aacA1         | ATG GGC ATC ATG GCC ACG TGT | 873 [38]           |                    |
| aacC2         | ATG CAT AGC CGG AAG GCA ATA AC | 861 [38]  |                    |
| aadA1         | ATG AGA GAG GGG GTG ATC G   | 792 [38]           |                    |
| aadB          | ATG GAC ACA ACG CAC TGC GC  | 534 [38]           |                    |
| aphA6         | ATG GAA TTT CCC AAT ATT ATT C | 781 [38]  |                    |
| armA          | AGG TTT TTT CCA ATG TGC AG  | 591 [57]           |                    |
| irtB          | CCC AAA CAG ACC GTC AAG GC  | 585 [57]           |                    |
| integron I    | GAC ATC ACG CAA GCA AAG    | Variable           |                    |

Table 1. Primers used for PCR and sequencing of drug resistance-associated genes from *K. pneumoniae* isolates.

(Accession number: 10.1371/journal.pone.0042280.t001)
rates of resistance to most drugs were much higher among ESBL positive isolates than ESBL negative isolates.

Drug Resistance Determinants of the K. pneumoniae Isolates

PCR and sequencing analysis were conducted for 223 K. pneumoniae isolates to analyze ESBL genes as well as drug resistance determinants conferring resistance to carbapenems, folate pathway inhibitors, fluoroquinolones, and aminoglycosides. The detailed information on the percentage of drug resistance-associated genes detected in K. pneumoniae isolates were summarized in Table 4 and Table 5. The percentage of isolates with 8 or more drug resistance-associated genes was 24.2% (n = 54). Among the beta-lactamase genes, the most frequently detected ones include: \( \text{bla}_{\text{CTX-M-10}} \) (80, 35.9%), \( \text{bla}_{\text{SHV-1}} \) (55, 24.7%), \( \text{bla}_{\text{SHV-11}} \) (47, 21.1%), \( \text{bla}_{\text{CTX-M-1}} \) (37, 16.6%), \( \text{bla}_{\text{CTX-M-11}} \) (37, 16.6%), \( \text{bla}_{\text{CTX-M-15}} \) (34, 15.2%) and \( \text{bla}_{\text{CTX-M-5}} \) (32, 14.3%). Except for \( \text{bla}_{\text{NDM}} \), all the other 4 examined carbapenemase genes including \( \text{bla}_{\text{KPC-2}} \), \( \text{bla}_{\text{IMP}} \), \( \text{bla}_{\text{VIM}} \), and \( \text{bla}_{\text{OXA-48}} \) were detected in this study. The prevalence of AmpC beta-lactamaes including \( \text{bla}_{\text{CMY-2}} \), \( \text{bla}_{\text{DHA-1}} \), and \( \text{bla}_{\text{FOX}} \) were 3.1%, 4.0% and 0.0%, respectively in this study. Among the 7 plasmid-encoded fluoroquinolone resistance-associated genes including \( \text{qnrA} \), \( \text{qnrB} \), \( \text{qnrC} \), \( \text{qnrD} \), \( \text{qnrS} \), \( \text{aac(6')-Ib-cr} \), and \( \text{qepA} \), the highest rates were observed for \( \text{qnrS} \) (58, 26.0%) and \( \text{aac(6')-Ib-cr} \) (53, 23.8%). In addition, \( \text{gyrA} \) gene mutations including T247A (Ser83Ile) (21, 9.4%), C248T (Ser83Phe) (15, 6.7%), and A260C (Asp87Ala) (16, 7.2%) were identified. No mutations were detected in \( \text{parC} \) gene. Among the aminoglycosides resistance-associated genes, the highest rates were observed for \( \text{aacC2} \) (73, 32.7%), \( \text{aacA4} \) (57, 25.6%), and \( \text{aadA1} \) (56, 25.1%). The prevalence of the plasmid-encoded 16 S rRNA methylases \( \text{armA} \) and \( \text{rmtB} \) were detected to be 5.8% and 3.6%, respectively. Class 1 integrons were detected in 47.5% (n = 106) of the isolates. In order to evaluate the correlation between phenotypic and genotypic drug resistance profiles, we also calculated the proportion of antibiotic resistance-associated genes among the phenotypic resistant isolates as well as the phenotypic susceptible isolates. We found that while some of the previously reported resistance-associated genes were indeed detected at relatively higher rates among corresponding phenotypic resistant

### Table 2. Demographic and clinical characteristics of the patients.

| Characteristics                  | Total n = 223 (%) | Patients infected with MDR isolates n = 137 (%) | Patients infected with XDR isolates n = 49 (%) | Patients infected with PDR isolates n = 4 (%) | Patients infected with other types of isolates n = 33 (%) | P value |
|----------------------------------|-------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|--------------------------------------------------------|---------|
| Gender                           |                   |                                               |                                               |                                               |                                                        |         |
| Male                             | 164 (73.5)        | 101 (73.7)                                    | 35 (71.4)                                     | 4 (100)                                       | 24 (72.7)                                              | 0.991   |
| Female                           | 59 (26.5)         | 36 (26.3)                                     | 14 (28.6)                                     | 0                                             | 9 (27.3)                                               |         |
| Age group, years                 |                   |                                               |                                               |                                               |                                                        | 0.400   |
| <18                              | 7 (3.1)           | 6 (4.4)                                       | 0                                             | 0                                             | 1 (3.0)                                                |         |
| 18–64                            | 64 (28.7)         | 39 (28.5)                                     | 11 (22.4)                                     | 2 (50.0)                                      | 12 (36.4)                                              |         |
| >64                              | 152 (68.2)        | 92 (67.2)                                     | 38 (77.6)                                     | 2 (50.0)                                      | 20 (60.6)                                              |         |
| Residence situation              |                   |                                               |                                               |                                               |                                                        | 0.862   |
| Beijing resident                 | 68 (30.5)         | 40 (29.2)                                     | 17 (34.7)                                     | 0                                             | 11 (33.3)                                              |         |
| Non-Beijing resident             | 155 (69.5)        | 97 (70.8)                                     | 32 (65.3)                                     | 4 (100.0)                                     | 22 (66.7)                                              |         |
| Hospital location                |                   |                                               |                                               |                                               |                                                        | 0.199   |
| Emergency room                   | 9 (4.0)           | 7 (5.1)                                       | 2 (4.1)                                       | 0                                             | 0                                                      |         |
| Intensive care unit              | 75 (33.6)         | 51 (37.2)                                     | 17 (34.7)                                     | 0                                             | 7 (21.2)                                               |         |
| Medical ward                     | 97 (43.5)         | 59 (43.1)                                     | 18 (36.7)                                     | 2 (50.0)                                      | 18 (54.5)                                              |         |
| Surgical ward                    | 42 (18.8)         | 20 (14.6)                                     | 12 (24.5)                                     | 2 (50.0)                                      | 8 (24.2)                                               |         |
| Sources of specimens             |                   |                                               |                                               |                                               |                                                        | 0.187   |
| Sputum                           | 168 (75.3)        | 108 (78.8)                                    | 34 (69.4)                                     | 3 (75.0)                                      | 23 (69.7)                                              |         |
| Urine                            | 14 (6.3)          | 7 (5.1)                                       | 7 (14.3)                                      | 0                                             | 0                                                      |         |
| Throat or nose swabs             | 21 (9.4)          | 11 (8.0)                                      | 2 (4.1)                                       | 0                                             | 8 (24.2)                                               |         |
| Catheters                        | 3 (1.3)           | 2 (1.5)                                       | 1 (2.0)                                       | 0                                             | 0                                                      |         |
| Blood                            | 10 (4.5)          | 4 (2.9)                                       | 3 (6.1)                                       | 1 (25.0)                                      | 2 (6.1)                                                |         |
| Puncture fluid                   | 1 (0.4)           | 1 (0.7)                                       | 0                                             | 0                                             | 0                                                      |         |
| Drainage fluid                   | 1 (0.4)           | 1 (0.7)                                       | 0                                             | 0                                             | 0                                                      |         |
| Pleural effusion                 | 2 (0.9)           | 1 (0.7)                                       | 1 (2.0)                                       | 0                                             | 0                                                      |         |
| Plus                             | 1 (0.4)           | 1 (0.7)                                       | 0                                             | 0                                             | 0                                                      |         |
| Bile                             | 2 (0.9)           | 1 (0.7)                                       | 1 (2.0)                                       | 0                                             | 0                                                      |         |
| ESBL                             |                   |                                               |                                               |                                               |                                                        | <0.001  |
| Positive                         | 101 (45.3)        | 54 (39.4)                                     | 42 (85.7)                                     | 4 (100.0)                                     | 1 (3.0)                                                |         |
| Negative                         | 122 (54.7)        | 83 (60.6)                                     | 7 (14.3)                                      | 0                                             | 32 (97.0)                                              |         |

doi:10.1371/journal.pone.0042280.t002
Table 3. Drug resistance rates of *K. pneumoniae* isolates.

| Antimicrobial category | Drugs* | Range (μg/mL) | MIC₅₀ (μg/mL) | MIC₉₀ (μg/mL) | ESBL positive n = 101 | ESBL negative n = 122 | Total n = 223 |
|------------------------|--------|---------------|---------------|---------------|----------------------|----------------------|--------------|
| Penicillins (99.6%, 222/223) | AMP    | ≤2–>32       | ≥32           | ≥32           | 101 (100)           | 121 (99.2)               | 222 (99.6)   |
| Antipseudomonal penicillins + beta-lactam inhibitors (22.4%, 50/223) | TZP    | ≤4–>128      | ≤4            | ≥128          | 29 (28.7)           | 21 (17.2)               | 50 (22.4)    |
| Penicillins + beta-lactam inhibitors (51.6%, 115/223) | SAM    | ≤2–>32       | 16            | ≥32           | 88 (87.1)           | 27 (22.1)               | 115 (51.6)   |
| 1st and 2nd generation cephalosporins (51.1%, 114/223) | CFZ    | ≤4–>64       | 32            | ≥64           | 96 (95.0)           | 18 (14.8)               | 114 (51.1)   |
| 3rd and 4th generation cephalosporins (49.3%, 110/223) | CRO    | ≤1–>64       | ≤1            | ≥64           | 95 (94.1)           | 15 (12.3)               | 110 (49.3)   |
| Cephamycins (68.6%, 153/223) | CTT    | ≤4–>64       | ≤4            | ≤4            | 43 (42.6)           | 110 (90.2)              | 153 (68.6)   |
| Carbapenems (7.2%, 16/223) | ETP    | ≤0.5–>8      | ≤0.5          | ≤0.5          | 5 (5.0)             | 11 (9.0)               | 16 (7.2)     |
| Monobactams (48.9%, 109/223) | IMP    | ≤1–>16       | ≤1            | ≥64           | 96 (95.0)           | 13 (10.7)               | 109 (48.9)   |
| Fluoroquinolones (40.4%, 90/223) | CIP    | ≤0.25–>4     | 1             | ≥4            | 63 (62.4)           | 27 (22.1)               | 90 (40.4)    |
| Aminoglycosides (49.3%, 110/223) | LVX    | ≤0.25–>8     | 1             | ≥8            | 63 (62.4)           | 27 (22.1)               | 90 (40.4)    |
| Folate pathway inhibitors (52.9%, 118/223) | TOB    | ≤1–>16       | ≤1            | ≥16           | 70 (69.3)           | 22 (18.0)               | 109 (48.9)   |
| Nitrofurantoin (85.2%, 190/223) | AMK    | ≤2–>64       | ≤2            | ≥16           | 39 (38.6)           | 15 (12.3)               | 54 (24.2)    |
|                  | FD     | ≤16–>512     | 64            | ≥512          | 89 (88.1)           | 101 (82.8)              | 190 (85.2)   |

*Abbreviation of drugs: AMP, Ampicillin; TZP, Piperacillin/Tazobactam; SAM, Ampicillin/Sulbactam; CFZ, Cefazolin; CRO, Ceftriaxone; CAZ, Ceftazidime; FEP, Cefepime; CTB, Cefotetan; ETP, Ertapenem; IMP, Imipenem; ATM, Aztreonam; CIP, Ciprofloxacin; LVX, Levofloxacin; GM, Gentamycin; TOB, Tobramycin; AMK, Amikacin; SXT, Trimethoprim-Sulfamethoxazole; FD, Furadantin.

[doi:10.1371/journal.pone.0042280.t003](https://doi.org/10.1371/journal.pone.0042280.t003)
isolates, some others were detected in very low proportion of the phenotypic resistant isolates. In addition, some of the resistance-associated genes were also detected in a sizable proportion of the phenotypic susceptible isolates.

Characteristics of Carbapenem-resistant *K. pneumoniae* Isolates

Among the 223 isolates, 16 were detected to be carbapenem-resistant and were used for further characterization. Most of the patients from whom the isolates obtained were male 87.5% (14/16) and aged patients (all were 56 years old or above). Among the 14 patients whose treatment outcome information was available, 6 died. Although carbapenemase genes were detected only in 7 of the 16 isolates, the majority of them exhibited resistance to a high number of drugs and contained a variety of corresponding drug resistance-associated genes. The proportion of MDR, XDR and PDR were 12.5% (n = 2), 62.5% (n = 10), 25.0% (n = 4), respectively. The more detailed characteristics of those carbapenem-resistant isolates are shown in Table S1.

Transmissibility of Drug Resistance of ESBL Positive MDR, XDR and PDR *K. pneumoniae* Isolates

Twelve *K. pneumoniae* isolates including 4 MDR, 4 XDR, and 4 PDR isolates were selected to test the natural transmissibility of antibiotic resistance by conjugation experiments. As shown in Table 6, the resistance to various drugs and the corresponding resistance-associated genes were transferred in all tested isolates, though to different extent. The most frequently transferred genes include *blaCTX-M-1* (6/7, 85.7%), *blaCTX-M-14* (4/5, 80.0%), *blaCTX-M-15* (3/4, 75.0%), *blaCTX-M-10* (2/3, 66.7%), *blaCTX-M-25* (2/2, 100%), *blaCTX-M-55* (2/2, 100%), *blaSHV-1* (3/3, 100%), *blaSHV-8* (2/2, 100%), *blaTEM-1* (2/2, 100%), *blaTEM-186* (1/1, 100%), *blaCMY-2* (1/1, 100%), *blaDHA-1* (1/1, 100%), *blaFOX* (1/1, 100%), *blaKPC-2* (1/1, 100%), *blaNDM-1* (1/1, 100%), *blaIMP* (1/1, 100%), *blaVIM* (1/1, 100%), *blaOXA-48* (1/1, 100%). Five transconjugants contained plasmids with the same size as those in their respective donors (TZSKP-1, 9, 15, 146, and

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**Table 4. Percentage of beta-lactamase antibiotics resistance-associated genes detected in *K. pneumoniae* isolates.**

| Target antimicrobial category | Resistance-associated genes | Resistance-associated genes detected in phenotypic resistant isolates, n/Na (%) | Resistance-associated genes detected in phenotypic susceptible isolates, n/Nb (%) | Resistance-associated genes detected in all isolates, n/Nc (%) |
|------------------------------|-----------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------|
| Antipseudomonal penicillins + beta-lactamase inhibitors, penicillins + beta-lactamase inhibitors, 1st and 2nd generation cephalosporins, 3rd and 4th generation cephalosporins, cephemycins (n = 204) | *blaCTX-M-1* | 35/204 (17.2) | 2/19 (10.5) | 37/223 (16.6) |
| *blaCTX-M-2* | 1/204 (0.5) | 1/19 (5.3) | 2/223 (0.9) |
| *blaCTX-M-3* | 31/204 (15.2) | 1/19 (5.3) | 32/223 (14.3) |
| *blaCTX-M-8* | 20/204 (9.8) | 2/19 (10.5) | 22/223 (9.9) |
| *blaCTX-M-9* | 18/204 (8.8) | 2/19 (10.5) | 20/223 (9.0) |
| *blaCTX-M-14* | 37/204 (18.1) | 0 | 37/223 (16.6) |
| *blaCTX-M-15* | 34/204 (16.7) | 0 | 34/223 (15.2) |
| *blaCTX-M-10* | 76/204 (37.3) | 4/19 (21.1) | 80/223 (35.9) |
| *blaCTX-M-25* | 2/204 (1.0) | 1/19 (5.3) | 3/223 (1.3) |
| *blaCTX-M-55* | 3/204 (1.5) | 0 | 3/223 (1.3) |
| *blaSHV-1* | 55/204 (27.0) | 0 | 55/223 (24.7) |
| *blaSHV-2* | 2/204 (1.0) | 0 | 2/223 (0.9) |
| *blaSHV-11* | 44/204 (21.6) | 3/19 (15.8) | 47/223 (21.1) |
| *blaSHV-45* | 15/204 (7.4) | 0 | 15/223 (6.7) |
| *blaTEM-1* | 19/204 (9.3) | 0 | 19/223 (8.5) |
| *blaTEM-186* | 3/204 (1.5) | 0 | 3/223 (1.3) |
| *blaCMY-2* | 7/204 (3.4) | 0 | 7/223 (3.1) |
| *blaOXA-48* | 9/204 (4.4) | 0 | 9/223 (4.0) |
| *blaFOX* | 0 | 0 | |
| Carbapenems (n = 16) | *blaKPC* | 0 | 0 | 1/1 (0.4) |
| *blaNDM* | 1/1 (6.3) | 0 | 1/1 (6.3) |
| *blaIMP* | 1/1 (6.3) | 0 | 1/1 (6.3) |
| *blaVIM* | 1/1 (6.3) | 0 | 1/1 (6.3) |
| *blaOXA-48* | 5/1 (3.1) | 0 | 5/1 (3.1) |

*<sup>a</sup>n/N<sub>a</sub>: No. of designated drug resistance-associated genes/No. of isolates resistant to the corresponding drugs.

*<sup>b</sup>n/N<sub>b</sub>: No. of designated drug resistance-associated genes/No. of isolates susceptible to the corresponding drugs.

<sup>c</sup>n/N<sub>c</sub>: No. of designated drug resistance-associated genes/No. of all isolates.

doi:10.1371/journal.pone.0042280.t004
208) (Figure 1). In addition, the transconjugant for TZSKP-82 harbored new plasmid with different size than that in the donor strain. The phylogenetic tree based on the MLST analysis results for the isolates is shown in Figure 2. Seven different STs were identified for those 12 isolates. Three isolates (TZSKP-1, 9, and 82) belonged to ST15, three isolates (TZSKP-13, 15, and 17) belonged to ST11, two isolates (TZSKP-228 and 245) belonged to ST218, and the rest of the isolates had unique STs. The epidemiological links were further determined for the patients from whom the clustered isolates were obtained.

**Discussion**

Worldwide emergence and dissemination of ESBL and carbapenemase genes among *Enterobacteriaceae*, especially in *K. pneumoniae* isolates, poses a considerable threat to public health. The major goal of this study was to evaluate the current situation and genetic background of drug-resistant *K. pneumoniae* isolates from patients in hospital settings. The highest and lowest resistance rates were observed for penicillins (99.6%) and carbapenems (7.2%), respectively. The rates of MDR, XDR and PDR isolates observed in this study are alarmingly high. This could cause difficulty in treating *K. pneumoniae*-associated infections since fewer and fewer effective drugs are available for treating those highly drug-resistant isolates. We also found that the proportion of MDR and other types of cases was significantly higher in patients with ESBL-negative isolates than that for XDR and PDR cases. This could be partially explained by the fact that the ESBL-positive isolates are normally resistant to many drugs, leaving only a few effective drugs available for treatment, which could lead to further resistance to those drugs. Indeed, this study further reveals that resistance to most of the drugs was found to be associated with ESBL positivity. Infections due to those ESBL positive and highly resistant strains are reported to be associated with higher morbidity and mortality rates [19–22], thus globally coordinated surveillance of epidemiology of those resistant isolates are warranted.

Another goal of this study was to evaluate the correlation between resistance phenotypes and the genetic determinants clinical *K. pneumoniae* isolates, so as to give a “snapshot” of the background of those resistant isolates. A striking feature of this study is the large number of antibiotic resistance-associated genes detected in the examined isolates. We also found that while some of the previously reported resistance-associated genes were indeed detected at relatively higher rates among corresponding phenotypic resistant isolates, some others were detected in very low proportion of the phenotypic resistant isolates, suggesting the existence of unknown drug resistance mechanisms such as reduced permeability of the outer membrane or up-regulated unknown efflux pumps in some clinical isolates [27,20]. In addition, some of

| Target antimicrobial category | Resistance-associated genes | Resistance-associated genes detected in phenotypic resistant isolates, n/Na (%) | Resistance-associated genes detected in phenotypic susceptible isolates, n/Nb (%) | Resistance-associated genes detected in all isolates, n/Nc (%) |
|-----------------------------|-----------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Folate pathway inhibitors (n = 118) | Dhfr | 52/118 (44.1) | 10/105 (9.5) | 62/223 (27.8) |
| Fluoroquinolones (n = 90) | qnrA | 1/90 (1.1) | 1/133 (0.8) | 2/223 (0.9) |
| | qnrB | 33/90 (36.7) | 6/133 (4.5) | 39/223 (17.5) |
| | qnrC | 1/90 (1.1) | 1/133 (0.8) | 2/223 (0.9) |
| | qnrD | 23/90 (25.6) | 12/133 (9.0) | 35/223 (15.7) |
| | qnrS | 22/90 (24.4) | 36/133 (27.1) | 58/223 (26.0) |
| | aac(6’)-Ib-cr | 45/90 (50.0) | 8/133 (6.0) | 53/223 (23.8) |
| | qepA | 1/90 (1.1) | 1/133 (0.8) | 2/223 (0.9) |
| | gyrA mutations | T247A(Ser83Ile) | 18/90 (20.0) | 3/133 (2.3) | 21/223 (9.4) |
| | | C248T(Ser83Phe) | 12/90 (13.3) | 3/133 (2.3) | 15/223 (6.7) |
| | | A260C(Asp87Ala) | 13/90 (14.4) | 3/133 (2.3) | 16/223 (7.2) |
| | parC mutations | None | None | None |
| Aminoglycosides (n = 110) | aacA4 | 50/110 (45.5) | 7/113 (6.2) | 57/223 (25.6) |
| | | aacC1 | 3/110 (2.7) | 0 | 3/223 (1.3) |
| | | aacC2 | 66/110 (60.0) | 7/113 (6.2) | 73/223 (32.7) |
| | | aadA1 | 49/110 (44.5) | 7/113 (6.2) | 56/223 (25.1) |
| | | aadB | 4/110 (3.6) | 0 | 4/223 (1.8) |
| | | aphA6 | 1/110 (0.9) | 0 | 1/223 (0.4) |
| | | armA | 13/110 (11.8) | 0 | 13/223 (5.8) |
| | | rmtB | 8/110 (7.3) | 0 | 8/223 (3.6) |

*a/N: No. of designated drug resistance-associated genes/No. of isolates resistant to the corresponding drugs.*

*b/N: No. of designated drug resistance-associated genes/No. of isolates susceptible to the corresponding drugs.*

*c/N: No. of designated drug resistance-associated genes/No. of all isolates.*

Table 5. Percentage of non-beta-lactamase antibiotics resistance-associated genes detected in *K. pneumoniae* isolates.
Table 6. Transmissibility of drug resistance of ESBL positive MDR, XDR and PDR K. pneumoniae isolates by conjugation.

| K. pneumoniae isolates | Resistance profile of K. pneumoniae isolates<sup>a,b</sup> | Resistance-associated genes detected in K. pneumoniae isolates<sup>b</sup> |
|------------------------|----------------------------------------------------------|--------------------------------------------------|
| MDR isolates           |                                                          |                                                  |
| TZSKP-9                | AMP, SAM, CFZ, CRO, CAZ, FEP, ATOM, CIP, LVX, SXT, FD   | blCTX-M-14, dhfr                                 |
| TZSKP-28               | AMP, SAM, CFZ, CRO, CAZ, FEP, ATOM, GM, TOB, SXT, FD   | blCTX-M-11                                      |
| TZSKP-40               | AMP, SAM, CFZ, CRO, CAZ, FEP, ATOM, GM, TOB, SXT, FD   | blCTX-M-14, blCTX-M-10, blTEM-11, blqnrS, aacC2  |
| TZSKP-208              | AMP, SAM, CFZ, CRO, CAZ, FEP, ATOM, SXT, FD             | blCTX-M-14, blCTX-M-10, blTEM-12, qnrS           |

| XDR isolates           |                                                          |                                                  |
| TZSKP-13               | AMP, SAM, CFZ, CRO, CAZ, FEP, ATOM, CIP, LVX, TOB, AMK, SXT, FD | blCTX-M-15, dhfr, qnrB, aacA4                    |
| TZSKP-15               | AMP, SAM, CFZ, CRO, CAZ, FEP, CTT, ETP, IMP, ATOM, CIP, LVX, GM, TOB, AMK, SXT, FD | blCTX-M-15, blCTX-M-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |
| TZSKP-146              | AMP, TZP, SAM, CFZ, CRO, CAZ, FEP, ATOM, CIP, LVX, GM, TOB, AMK, SXT, FD | blCTX-M-15, blCTX-M-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |
| TZSKP-228              | AMP, TZP, SAM, CFZ, CRO, CAZ, FEP, CTT, ETP, JPM, ATOM, TOB, AMK, SXT | blCTX-M-10, blTEM-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |

| PDR isolates           |                                                          |                                                  |
| TZSKP-1                | AMP, TZP, SAM, CFZ, CRO, CAZ, FEP, CTT, ETP, IMP, ATOM, CIP, LVX, GM, TOB, AMK, SXT, FD | blCTX-M-10, blTEM-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |
| TZSKP-17               | AMP, TZP, SAM, CFZ, CRO, CAZ, FEP, CTT, ATOM, CIP, LVX, GM, TOB, AMK, SXT, FD | blCTX-M-10, blTEM-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |
| TZSKP-82               | AMP, TZP, SAM, CFZ, CRO, CAZ, FEP, CTT, ETP, JPM, ATOM, CIP, LVX, GM, TOB, AMK, SXT, FD | blCTX-M-10, blTEM-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |
| TZSKP-245              | AMP, TZP, SAM, CFZ, CRO, CAZ, FEP, CTT, ETP, JPM, ATOM, CIP, LVX, GM, TOB, AMK, SXT, FD | blCTX-M-10, blTEM-10, blTEM-11, blqnrB, qnrB, aacA4, aadA1 |

<sup>a</sup>Abbreviation of drugs: AMP, Ampicillin; TZP, Piperacillin/Tazobactam; SAM, Ampicillin/Sulbactam; CFZ, Cefazolin; CRO, Ceftriaxone; CAZ, Ceftazidime; FEP, Cefepime; CTT, Eftotetan; ETP, Ertapenem; IMP, Imipenem; ATOM, Aztreonam; CIP, Ciprofloxacin; LVX, Levofloxacin; GM, Gentamycin; TOB, Tobramycin; AMK, Amikacin; SXT, Trimethoprim-Sulfamethoxazole; FD, Furadantin.

<sup>b</sup>The ones found in both donor strains and transconjugants were underlined to demonstrate the difference.

doi:10.1371/journal.pone.0042280.t006

Figure 1. Analysis of plasmid DNA from K. pneumoniae parental donor strains (Designated D) and derived E. coli transconjugants (Designated T) by agarose gel electrophoresis. Lanes: 1, molecular marker (Hind III digest DNA Marker, TaKaRa Code: D3403A); 2, TZSKP-1; 3, Transconjugant from TZSKP-1; 4, TZSKP-9; 5, Transconjugant from TZSKP-9; 6, TZSKP-13; 7, Transconjugant from TZSKP-13; 8, TZSKP-15; 9, Transconjugant from TZSKP-15; 10, TZSKP-17; 11, Transconjugant from TZSKP-17; 12, TZSKP-28; 13, Transconjugant from TZSKP-28; 14, TZSKP-40; 15, Transconjugant from TZSKP-40; 16, TZSKP-82; 17, Transconjugant from TZSKP-82; 18, TZSKP-146; 19, Transconjugant from TZSKP-146; 20, TZSKP-208; 21, Transconjugant from TZSKP-208; 22, TZSKP-228; 23, Transconjugant from TZSKP-228; 24, TZSKP-245; 25, Transconjugant from TZSKP-245.

doi:10.1371/journal.pone.0042280.g001
the resistance-associated genes were also detected in a sizable proportion of the phenotypically susceptible isolates, suggesting that individual resistance gene alone is not sufficient to cause resistance phenotype and only when some of them were accumulated can the resistance become detectable in the clinical isolates.

Rapid detection of genetic determinants associated with drug resistance in clinical *K. pneumoniae* isolates is crucial for appropriate antimicrobial therapy and infection control measures. We detected relatively high percentage of previously reported genes associated with resistance to beta-lactams [2,10,29–32], fluoroquinolones [28,33–37], aminoglycosides [38,39], and folate pathway inhibitors [15] in *K. pneumoniae* isolates. CTX-M-type beta-lactamase genes (such as *bla*CTX-M-14 and *bla*CTX-M-15) have been reported to be prevalent worldwide [40–42]. For example, a recent study from China reported that among the 21 *K. pneumoniae* isolates from 1270 specimens collected in a prospective multi-center study in eight teaching hospitals in China from June to December in 2007, 3 were detected to have *bla*CTX-M-14 (3, 14.3%) [40]. Another study conducted in Scotland showed that 16 of the 219 (7.3%) clinical isolates of *K. pneumoniae* collected in 2006 and 2007 at the Royal Infirmary of Edinburgh, Scotland had *bla*CTX-M-15 [41]. In the present study, the highest rate of CTX-M-type beta-lactamase genes was observed for *bla*CTX-M-10 (35.9%), followed by *bla*CTX-M-1 (16.6%), *bla*CTX-M-14 (16.6%), and *bla*CTX-M-15 (15.2%). The rates of the non-ESBL SHV-type beta-lactamase genes *bla*SHV-1 and *bla*SHV-11 genes were 24.7% and 21.1%, respectively in this study, which were relatively lower compared to that reported by some previous studies conducted in other regions. For example, according to a study conducted in Korea, the rates of the *bla*SHV-1 and *bla*SHV-11 genes among *K. pneumoniae* isolates collected from May to July, 2002 were 35% (30/142) and 62% (62/142), respectively [43]. Another study from Brazil reported that 55.8% (29/52) of the *K. pneumoniae* isolates collected in Recife, PE, Brazil during 1998 to 2005 harbored the *bla*SHV genes [44]. Thus, the prevalence of some beta-lactamase genes such as the *bla*SHV genes could be greatly variable geographically and timewise.

The more recently reported carbapenemases genes such as *bla*IMP, *bla*VIM, *bla*NDM, plasmid-mediated clavulanic acid-inhibited class A beta-lactamases genes such as *bla*KPC, and the class D beta-lactamase gene *bla*OXA-48 were rarely detected or undetected in this study. Carbapenemases increasingly have been reported in *Enterobacteriaceae* in the past decade. KPC carbapenemases have been reported in the United States and then worldwide [6,8]. VIM and IMP metallo-beta-lactamases also have been reported in many regions of the world, with a higher prevalence in southern Europe and Asia [1,5,45–47]. Carbapenemases of the oxacillinase-48 type (OXA-48) have been identified mostly in Mediterranean and European countries and in India [48,49]. Although the world-alarming New Delhi metallo-beta-lactamase-1 (NDM-1) was not detected in this study, it has been detected worldwide since it was first identified in India and its variants have emerged [13,50–52]. Thus resistance caused by those recently emerging beta-lactamases is still worrisome and needs continuous monitoring. The association of alterations in *gyrA* (gene encoding for GyrA subunit of DNA gyrase) and *parC* (gene encoding for ParC subunit of DNA topoisomerase IV) with fluoroquinolone resistance in *K. pneumoniae* is still not clear. Some studies suggested that in *K. pneumoniae*, DNA gyrase A is a primary target of quinolones and that ParC alterations play a complementary role in the development of higher-level fluoroquinolone resistance [53,54], while a study reported that hypermutation in *K. pneumoniae* is uncommon and does not contribute to accumulation of *gyrA* mutations or directly to ciprofloxacin resistance [55]. We identified 3 types of *gyrA* mutations including the previously reported C248T (Ser83Phe)
and A260C (Asp87Ala) [53] and the unreported T247A (Ser87Ile). No mutations in parC were detected in this study. The plasmid-encoded 16 S rRNA methylases armA and mtdB has emerged as a new mechanism of resistance to aminoglycosides, and the concomitant presence of armA or mtdB with blactX-M type beta-lactamase genes, especially the group 1 (CTX-M-3 and CTXM-15) or group 9 (CTX-M-14), among amikacin-resistant ESBL-producing K. pneumoniae isolates was reported in Taiwan and Belgium [36,37]. In this study, both armA and mtdB were detected (5.8% and 3.6%, respectively). One isolate was found to harbor both armA and mtdB genes, and consistent with previous reports, the armA and mtdB genes were coexist with at least one of the blactX-M type beta-lactamases tested in this study.

Our study further confirmed the notion that patients infected with carbapenem-resistant K. pneumoniae isolates normally have worse treatment outcome. In addition, the conjugation results suggest that certain ESBL genes (such as blactX-M-14), aac(6')-Ib-cr and armA were frequently co-transmitted and co-selected in MDR, XDR and PDR isolates and can be naturally transferred to susceptible E. coli strains by conjugation. Five transconjugants contained plasmids with the same size as those in their respective donors. Nevertheless, plasmids of the same sizes found in both donor and recipient isolates could not guarantee that the resistance transfer was plasmid-mediated. A subsequent DNA-DNA hybridization experiment with probes made by the respective resistance genes is warranted to show that the plasmids of the same sizes do carry the same resistance genes. On the other hand, we noticed that plasmids were not identified in some of the donor and recipient isolates. This could be explained by the existence of some other non-plasmid-mediated mechanisms involved in the occurrence and transfer of drug resistance in those isolates. For example, the drug resistance-associated genes could be carried on chromosomally located transposons and integrons. Although the isolate TZSKP-28 was detected to be ESBL-positive, only the non-ESBL blasmHV-11 gene was detected in it. After conjugation experiments, the recipient strain became multidrug resistant and again only the blasmHV-11 was detected. We also noticed that the beta-lactamase genes found in the transconjugants of the isolates TZSKP-1 and TZSKP-40 (blasmHV-11 or blaseV-41) were not ESBLs, either. This result suggested that some other mechanisms may be involved in causing the ESBL positivity and MDR phenotypes in those isolates. As horizontal transmission event can result in the acquisition of multidrug resistance by wild-type strains, thus this could presumably contribute to the rapid increase in the prevalence of multidrug resistance among clinical bacteria. Another important aspect for infection control is to know whether there is a clonal spread among the highly drug-resistant isolates. Relatively diverse genotypes were identified for those 12 isolates used in conjugation analysis by MLST analysis. Three clusters (which belonged to ST15, ST11, and ST218, respectively) each consisted of 2 or 3 isolates were detected and the epidemiological links were not observed for the patients from whom the clustered isolates were obtained. Further investigation of the transmission patterns of a larger sample of drug-resistant K. pneumoniae isolates by MLST analysis is warranted and which is currently underway. Emerging plasmid-encoded ESBLs and carbapenemases are increasingly reported worldwide [58]. Carbapenemase production encoded by genes located on mobile genetic elements is typically accompanied by genes encoding resistance to other drug classes, and are frequently located on the same mobile DNA elements such as integrons, which act as bacterial recombination systems that mediate the capture and expression of gene cassettes and are considered as the primary mechanism for antibiotic resistance gene acquisition among bacteria and are frequently associated with transposons and conjugative plasmids (http://integrall.bio. ua.pt 2009) [59,60]. In this study, class 1 integrons were detected in a relatively high percentage of the isolates. Further sequencing analysis of class 1 integrons and gene cassette arrays is currently undergoing in the lab.

In summary, our results indicate that there is a high prevalence and possible transmission of MDR, XDR and PDR K. pneumoniae isolates among hospitalized patients. In addition, our data give a “snapshot” of the complex genetic background responsible for drug resistance in those highly drug-resistant K. pneumoniae isolates. Thus our study demonstrate that a high degree of awareness and monitoring of those drug resistance determinants are urgently needed in order to better control the emergence and transmission of drug-resistant K. pneumoniae isolates in hospital settings.

Supporting Information

Table S1 Characteristics of carbapenem-resistant K. pneumoniae isolates.

| DOC |

Acknowledgments

We are grateful to Professor George A. Jacoby from Lahey Clinic Medical Center, for kindly sending us the E. coli J53 AzR for the conjugation experiments.

Author Contributions

Conceived and designed the experiments: CHL GFG. Performed the experiments: CHL BL YY QW LT. Analyzed the data: CHL BL YY QW PCYW LT HJ GFG. Contributed reagents/materials/analysis tools: CHL. Performed the experiments: CHL BL YY QW LT. Analyzed the data: CHL BL YY QW PCYW LT HJ GFG. Wrote the paper: CHL. Read and approved the final manuscript: CHL BL YY PCYW QW LT HJ GFG.

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