Molecular epidemiologic factors contributing to quinolone resistance in clinical multidrug-resistant *Klebsiella pneumoniae* isolates from Shanghai, China

Yan Wang (wangyan_hf@126.com)
Fudan University https://orcid.org/0000-0002-8457-5287

Guoping Cai
Fudan University Jinshan Hospital

Jinan Zhang
Shanghai Jiankang Medical University

Xiaogang Xu
Fudan University Huashan Hospital Institute of Antibiotics

Hongzhou Lu
Fudan University Affiliated Public Health Clinical Center: Shanghai Public Health Clinical Center

Research article

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Abstract

Background

The soaring quinolone-resistance rate of Klebsiella pneumoniae, a common pathogen in immunocompromised individuals, has seriously undermined the wide applications of antimicrobials of this class. This study aimed to investigate the emerging key contributors to quinolone-resistance in multidrug resistant K. pneumoniae (MDR-KP) isolates from a clinical setting with continuing point-source infection outbreaks in Shanghai, China.

Results

Between January and March 2017, a total of 34 K. pneumoniae isolates, including 30 carbapenem-resistant K. pneumoniae (CRKP), were selected and characterized from a teaching hospital participating in an ongoing Bacterial Resistance Surveillance Project in Shanghai, China. Two predominant high-risk CRKP clones, ST11-wzi64 and ST15-wzi19/wzi24, caused three point-source nosocomial outbreaks in intensive care unit and/or neurosurgery department potentially by respiratory-route, promoting the co-selection and evolution of multidrug-resistant determinants. Multiple quinolone resistance-determining region (QRDR) mutations occurred in isolates of ST15 (S83F, D87A; S80I), ST11 (S83I, D87G; S80I), and ST218 (D87A; S80I). Plasmid-mediated quinolone resistance determinants, qnrS1, aac(6')-Ib-cr, oqxAB, were detected in 32 (94.1%) isolates alone or in combination, spreading accompanied with β-lactamases (mainly, KPC-2-type carbapenemase and CTX-M-type extended-spectrum β-lactamase), 16S rRNA methylases (ArmA and RmtB), and putrescine ABC transporter permease (PotI) variants, independently of QRDR-mutations. AcrR, AcrAB transcriptional repressor, was insertion-inactivated by IS5-transposase in isolates of ST11. Thirteen ompK36 variants associated with specific ST (n=7) and wzi-allele (n=9) clustered into 10 (sub)lineages in the phylogenetic tree possibly affecting the MDR phenotype and the infection outcome of isolates. Isolates of ST11, ST15, and ST218 had frameshift disruptions in OmpK35 coupled with specific GD-insertion at position 134-135 in OmpK36, all showing distinct microevolution clusters of ompK36 genotypes. Seven quinolone-susceptible isolates kept the porin genes integral, including two each CRKPs of ST13-wzi74 (carbapenemase KPC-2 and NDM-1-coproducers) and ST65-wzi72.

Conclusions

Under selective pressures, accumulation of mutations of three types (QRDR, AcrR, OmpK36/OmpK35) and acquisition of resistance-conferring genes has been continuously contributing to quinolone-resistance in clinical MDR-KP isolates, reinforcing the importance of ongoing epidemiologic surveillance on the evolution and transmission of these isolates. Our findings provided detailed mechanistic analyses and epidemiologic implications for further infection control and antibiotic stewardship initiatives.

Full Text

This preprint is available for download as a PDF.

Figures

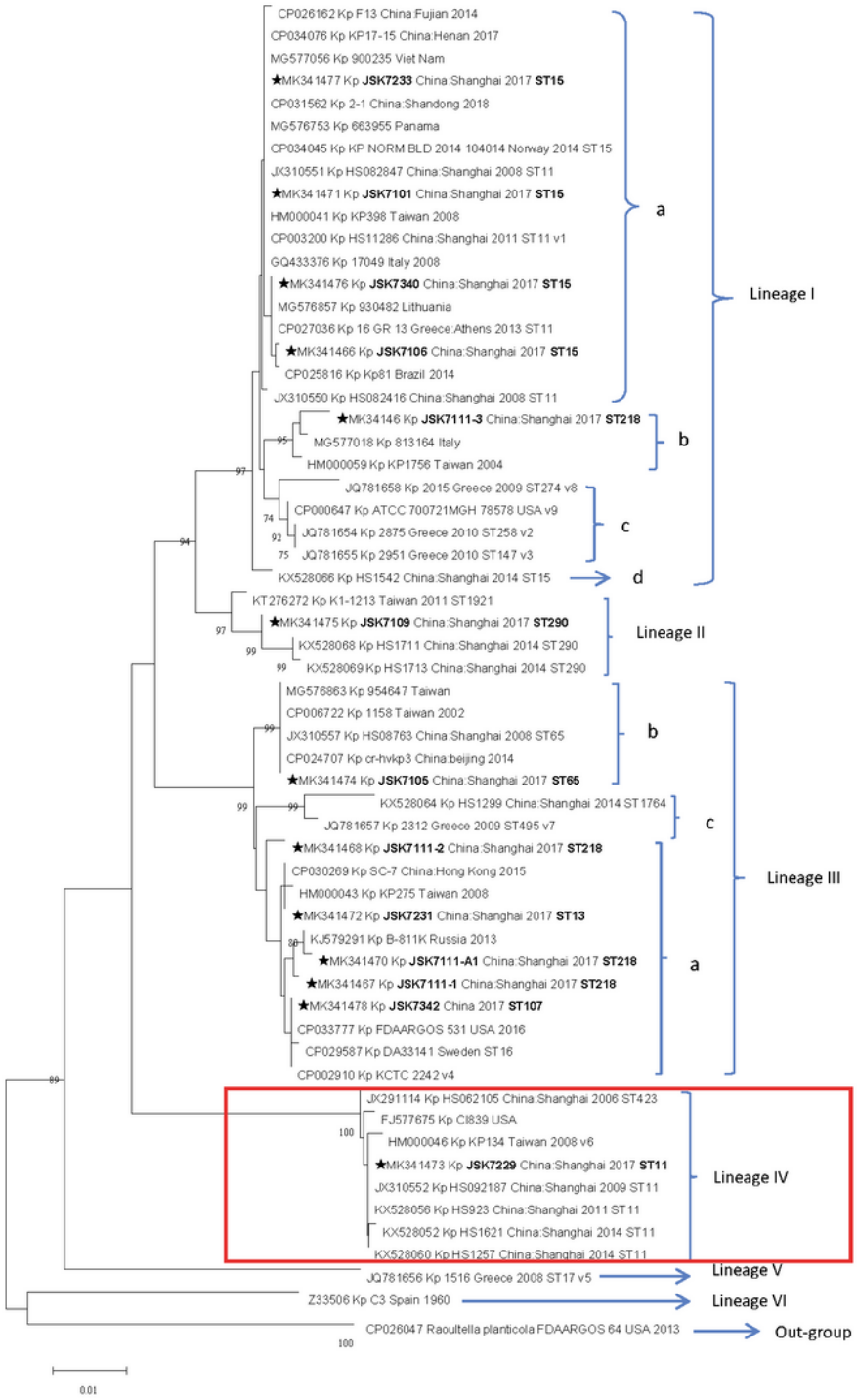

Figure 1

Phylogenetic analysis based on the coding sequences of ompK36 genes for K. pneumoniae isolates. The dendrogram was constructed using neighbor-joining method by MEGA program version 7.0. Bootstrap resampling (1000 replications) was used, and bootstrap values ≥70% were shown. Scale bar represents nt substitutions per site. Reference strains were named in reference to the accession-number, strain name, country of origin, isolation-year, and MLST. denotes K. pneumoniae isolates in the present study (GenBank accession number MK341466-MK341478) with its common name and ST shown in bold. The ompK36 for a Raoultella planticola isolate (CP026047) was taken as an out-group. The red box indicates the phylogenetic cluster position of isolates ST11 (Lineage IV).

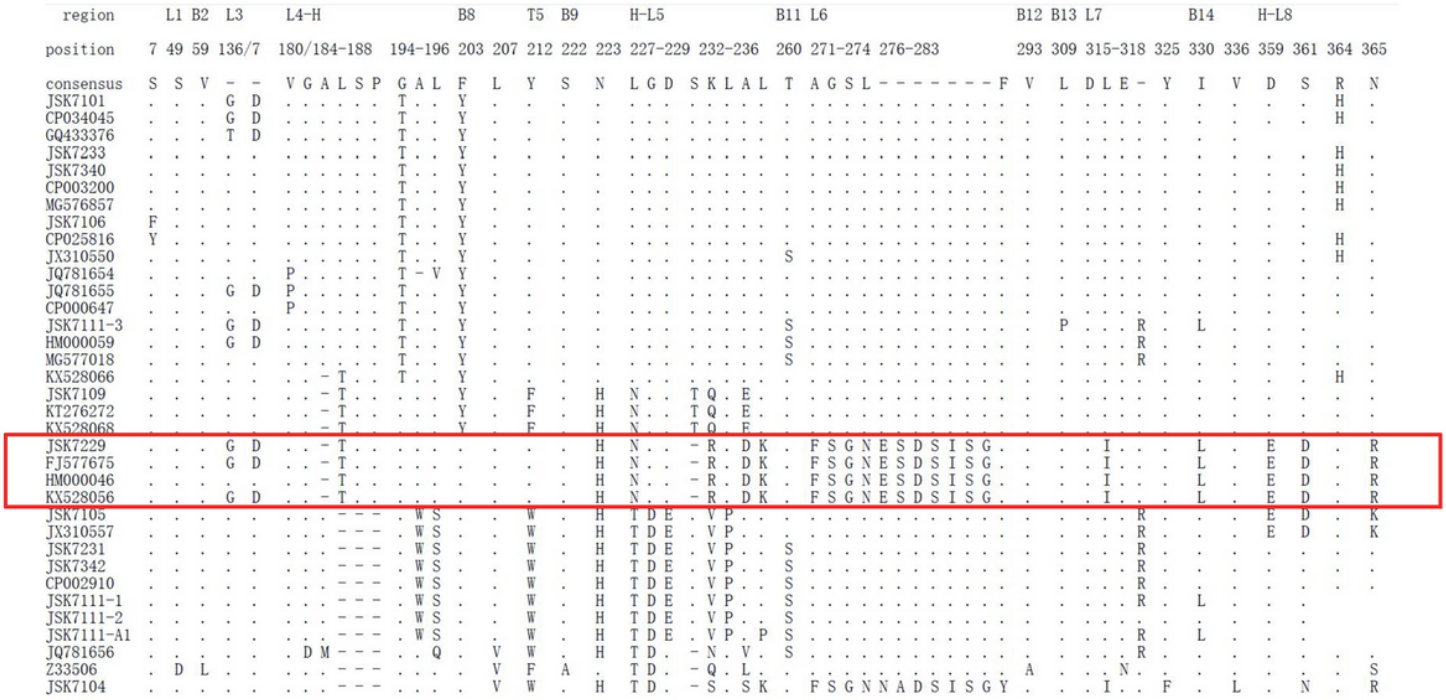

Figure 2

Comparison and alignment of Ompk36 porin regions from the representative of K. pneumoniae sample and reference isolates in Figure 1. The differentiated regions included Loops (L), beta-strands (B), turns (T) and alpha-helix (H). The consensus protein sequence was established employing the most common aa at each position in the present study. Amino acids are represented by standard single-letter codes and deletion in a site is indicated by a dash (-); those sites identical to the consensus sequence are indicated with dots. The numbers along the sequence indicated aa positions. Each sequence was designated as in the phylogenetic tree. The red box indicates the substitutions and insertions in loops of L3,L4,L5,L6,L7, and L8 for ST11 strains.

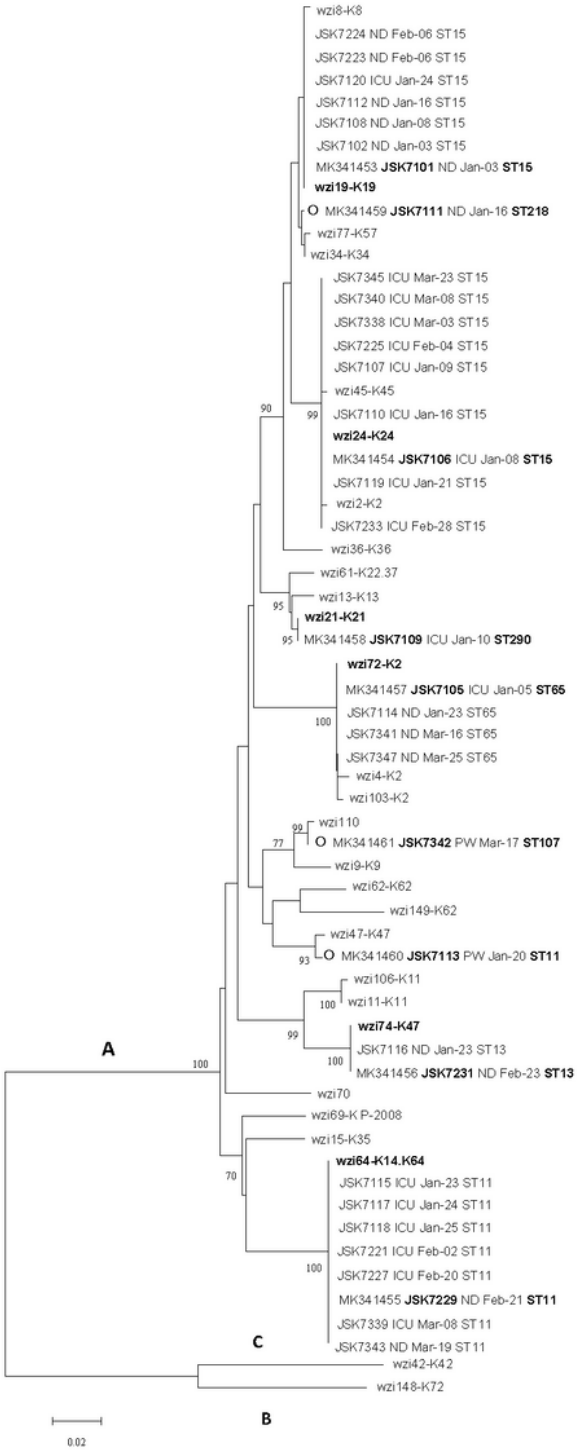

Figure 3

MLST and wzi-genotyping of 30 K. pneumonia dinical isolates. The dendrogram of wzi-alleles (0474) was built using neighbor-joining method by MEGA program version 7.0. Bootstrap resampling (1000 replications/ was used, and bootstrap values 770% were shown. Scale bar represents nt substitutions per site. Each known wzi-allele number is followed by the corresponding capsular (K) type, with dots separating several K types indicating cross-reactions. The three main branches (A, B, and C) are labeled.

The representative wzi sequences (GenBank accession number MK301453-MK301061) of sample isolates from the present study and their identical wzi-alleles in the same cluster were shown in bold. 0 denotes the new wzi variants without known allele-number. The common names of isolates were followed by ward of origin, isolation-date, and MLST. ND, Neurosurgery Department; PW, Pediatric Ward.

Supplementary Files

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- YanWangSupplementaryMaterial.pdf