Associations of the major international high-risk resistant clones and virulent clones with specific *ompK36* allele groups in *Klebsiella pneumoniae* in Taiwan

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Abstract

This study was conducted to investigate the association between *ompK36* variants and international high-risk clones in *Klebsiella pneumoniae*. Fifty-nine sequence types (STs) divided into four *ompK36* allele groups (groups A to D) were identified among 185 *K. pneumoniae* isolates. The major high-risk clones (29 ST11, 13 ST15, 7 ST37 and 1 ST147 isolates) were assigned to group A, while 6 STs (15 ST23, 2 ST65, 3 ST86, 1 ST163, 1 ST373 and 2 ST375 isolates) associated with pyogenic liver abscess were assigned to group C. The genotyping assay developed in this study may be useful for screening of epidemic STs.

Keywords: High-risk clones, *Klebsiella pneumoniae*, multilocus sequence typing, pyogenic liver abscess, sequence types
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Antibiotic-resistant bacterial clones with high interhost transmission and colonization efficiency may play an important role in the dissemination of antimicrobial resistance [1]. Several international high-risk resistant clones of *Klebsiella pneumoniae* have been identified by multilocus sequence typing (MLST) [1]. The major epidemic clones belong to sequence type (ST) 11 (ST11), ST15, ST147 and ST258 [1–9]. ST11 and ST258 are included in clonal group 258 (CG258), while ST15 and ST147 belong to two different CGs. ST11, ST15 and ST37 are the most common STs among carbapenem-nonsusceptible *K. pneumoniae* isolates in Taiwan [6].

The OmpK36 porin contributes to carbapenem susceptibilities and virulence in *K. pneumoniae* [10–12]. The correlation of OmpK36 variants with specific STs has been described [13]. It is therefore likely that sequence heterogeneity of *ompK36* is driven by selective pressures created by both host immunity and antimicrobial use. The present study was conducted to investigate the relationship between *ompK36* types and high-risk clones in *K. pneumoniae* in Taiwan.

Firstly, MLST was performed as described by Diancourt *et al.* [14] using 35 previously characterized *K. pneumoniae* isolates with decreased carbapenem susceptibilities and intact *ompK36* sequences (Table 1) [15–17]. Twelve STs were obtained, of which ST11 was the most common (16 isolates, 45.7%). Nucleotide sequences of *ompK36* were compared using the GCG SeqWeb software (University of Wisconsin Biotechnology Center, Madison, WI, USA), and multiple sequence alignments and a phylogenetic tree were generated using the Pileup and Growtree programs, respectively. Thirteen *ompK36* alleles were obtained; they were divided into four clusters, designated groups A, B, C and D (Fig. 1a). The major differences between the allele groups were located between nucleotide position 500 and 1000 in the coding region (Online Supplemental Fig. S1). Groups A, B, C and D accounted for 68.6%, 2.9%, 17.1% and 11.4% of the 35 isolates, and included 5, 1, 4 and 2 STs, respectively. All isolates of high-risk clones ST11, ST15 and ST37 belonged to group A. Pulsed-field gel electrophoresis was performed as described [16]. The result was consistent with the MLST analysis and showed genetic unrelatedness among isolates of different CGs with similar *ompK36* sequences (Fig. 1b).

A PCR-based method using *ompK36*-targeted group-specific primer pairs was then developed, and 69 previously characterized extended-spectrum β-lactamase (ESBL)-producing and/or AmpC-producing *K. pneumoniae* isolates from 7 medical centres were examined [18]. The primer sequences and amplicon sizes are as follows: 5′-GAAGGCGCTCTGTCTCCTA-3′ and 5′-TGCCATCATAGATGTCATAGG-3′ for group A, 97 bp; 5′-CGGTCGTGGCGCGCAGAAA-3′ and 5′-GGTTGTTCTGATCGTCGGTA-3′ for group B, 125 bp; 5′-CAACAACGGTCGTGGTTGGA-3′ and 5′-CCCAGTGCCGGAACACTATT-3′ for group C, 144 bp; 5′-GAAGGTACTTCTCCGACCAA-3′ and 5′-AATCAGATTCTCCGTTGCCG-3′ for group D, 283 bp.

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TABLE 1. Results of *ompK36* typing, β-lactamase typing and multilocus sequence typing for 81 *Klebsiella pneumoniae* isolates collected from the National Cheng Kung University in 2010

ompK36 group (no. of isolates)	Extended-spectrum β-lactamases or AmpC	ST (no. of isolates)
A (14)	DHA-1	ST11 (1)
	DHA-1	ST15 (1)
	SHV, DHA-1	ST15 (1)
	CTX-M-15	ST37 (1)
	SHV	ST147 (1)
	SHV	ST152 (1)
	SHV	ST1440 (2)
	Negative	ST15 (1), ST661 (1), ST950 (1), ST1536 (1), untypeable (2)
B (9)	CTX-M-15	ST711 (1)
		ST361 (1), ST605 (1), ST929 (1), ST1180 (1), untypeable (4)
C (39)	SHV, DHA-1	ST1 (1)
	SHV, DHA-1	ST39 (1)
	SHV	ST39 (1)
	SHV	ST48 (1)
	CTX-M-3	Untypeable (1)
	Negative	ST23 (13), ST25 (1), ST35 (1), ST39 (1), ST43 (1), ST65 (2), ST86 (3), ST373 (1), ST375 (2), ST660 (1), ST1049 (2), untypeable (6)
D (17)	SHV	ST133 (2)
	SHV, DHA-1	ST196 (1)
	Negative	ST36 (2), ST107 (1), ST133 (1), ST268 (2), ST298 (1), ST397 (1), ST420 (1), ST528 (1), ST776 (1), ST873 (1), ST1027 (1), ST1590 (1), untypeable (2)
Untypeable (2)	Negative	Untypeable (2)

ST, sequence type.

Simplex PCR experiments were performed under the PCR conditions as follows: 1 cycle of 94°C for 10 min; 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 60 seconds, with a final extension at 72°C for 10 minutes. PCR conditions were the same for all primer pairs. Of the 69 isolates, 35 (50.7%), 6 (8.7%), 23 (33.3%) and 5 (7.2%) isolates were assigned to groups A, B, C and D, respectively. Twelve ST11, 7 ST15, 3 ST37, 1 ST273, 3 ST395, 2 ST709 and 2 ST833 isolates were included in group A. The ST11, ST15, and ST37 isolates were collected from 5, 4 and 2 hospitals, respectively. Group B comprised 3 ST17, 1 ST414, 1 ST1465 and 1 ST1535 isolates. In group C, 10 STs (ST1, ST23, ST25, ST35, ST39, ST42, ST48, ST101, ST163 and ST416) were identified, of which each included 1 or 2 isolates. Group D comprised 1 ST321, 2 ST378, 1 ST528 and 1 ST655 isolates. Currently available alleles or STs were not obtained for 7 isolates.

The prevalence of each *ompK36* group among 81 *K. pneumoniae* bloodstream isolates collected at the National Cheng Kung University Hospital in 2010 was determined by the PCR-based *ompK36* typing tests. Phenotyping and genotyping of β-lactamases were performed as described previously [15–17]. Fourteen (17.3%), 9 (11.1%), 39 (48.1%) and 17 (21.0%) of the 81 isolates belonged to groups A, B, C and D, respectively, and 2 isolates could not be typed. The MLST analysis showed 41 STs and 13 unassignable types. The major international clones (ST11, ST15, ST37 and ST147) were uncommon (6 isolates, 7.4%). The virulent clone ST23 in group C was the most common ST (n = 13). Seventeen (21.0%) of the 81 isolates carried bla_{ESBL}, bla_{DHA-1} (6 isolates) or both (4 isolates), and groups A, B, C and D accounted for 8 (47.1%), 1 (5.9%), 5 (29.4%) and 3 (29.4%) of the 17 isolates, respectively.

In summary, a total of 59 currently available STs were identified, among which the major international high-risk clones (29 ST11, 13 ST15, 7 ST37 and 1 ST147 isolates) were grouped together by *ompK36* typing. Six STs reported to be associated with pyogenic liver abscess [19,20] were detected, and of note, all isolates of these STs (15 ST23, 2 ST65, 3 ST86, 1 ST163, 1 ST373 and 2 ST375 isolates) were assigned to the other *ompK36* allele group.

In conclusion, this study demonstrated the association of the major high-risk resistant clones and virulent STs with specific *ompK36* allele groups. The association might result from the occurrences of convergent evolution driven by the selective pressure created by antimicrobial use and host factors. Active surveillance of the epidemic resistant clones followed by appropriate infection control measures is needed to prevent the spread of antimicrobial resistance, and the *ompK36*-targeted PCR method developed in this study may be useful for screening of epidemic clones. The major high-risk resistant clones were uncommon. The use of the PCR screening method should therefore be cost-effective despite the lack of specificity, and subsequent sequencing of one or two housekeeping genes for MLST should reduce the number of isolates for complete MLST analysis further. Further large-scale studies are needed to confirm the association identified by *ompK36* typing and the usefulness of the typing method.

Conflict of interest

None declared.

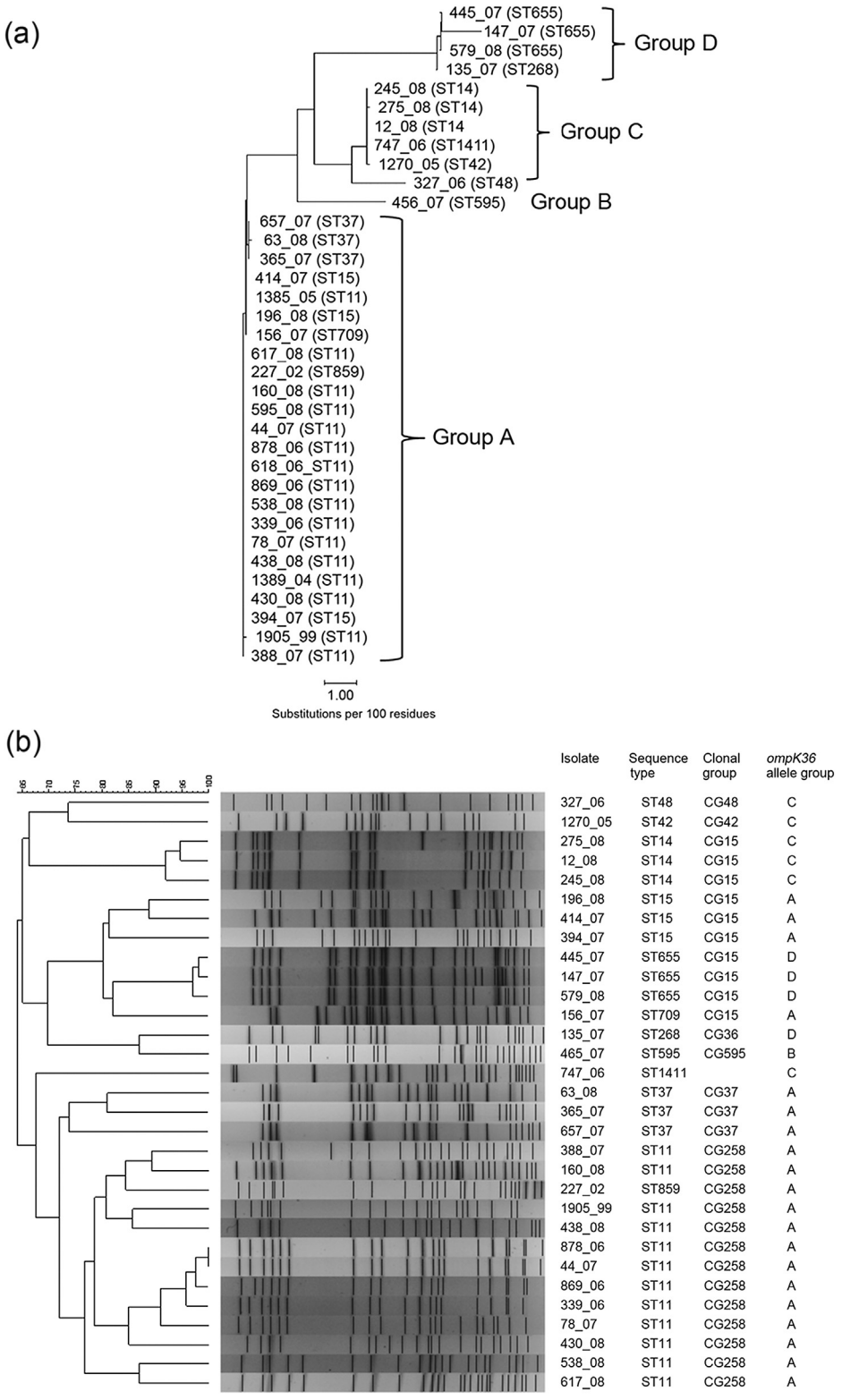

FIG. 1. Phylogenetic analysis of 35 previously characterized *K. pneumoniae* isolates with decreased carbapenem susceptibilities. (**a**) Phylogenetic tree based on nucleotide sequences of *ompK36* genes. The unrooted dendrogram was obtained with Growtree with uncorrected distances and neighbor joining. (**b**) Pulsed-field gel electrophoresis of *Xba*I-digested genomic DNA. Four isolates were not typeable probably due to DNA lysis. The profiles were compared using the BioNumerics program (Bio-Rad Laboratories). A dendrogram was generated from the distance matrix by the unweighted pair-group method with arithmetic mean, and genetic relatedness was calculated based on the Dice coefficient.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2015.01.002.

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