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Original Article

Evaluation of *ompK36* allele groups on clinical characteristics and virulence features of *Klebsiella pneumoniae* from bacteremia

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Abstract *Background/purpose:* This study investigated the implications of *ompK36* allele groups on clinical and microbiological features of patients with *Klebsiella pneumoniae* bacteremia.
Methods: A total of 80 K. *pneumoniae* bloodstream isolates were collected and then divided into four *ompK36* allele groups. Clinical characteristics, bacterial antibiotic resistance and virulence determinants were analyzed, including resistance and virulence genes, hypermucoviscosity phenotype, K capsule serotypes, biofilm formation, serum killing, neutrophil phagocytosis, and mouse lethality studies.
Results: 78 isolates were classified into four *ompK36* variants, designated groups A (34), B (6), C (26), and D (12), respectively; 2 isolate was untypeable. OmpK36 group C isolates carried higher frequencies of K1/K2 capsule serotypes, hypermucoviscosity phenotype, *rmpA* gene, *allS* gene, *iroB* gene, *aerobactin* gene, or *rmpA2* gene than non-C group isolates. OmpK36 group C isolates were significantly more virulent, as higher serum resistance, higher anti-phagocytosis and higher mouse lethality, than OmpK36 non-C group isolates, except for similar biofilm formation capability. The K20 isolates probably has low expression rates of rmpA and rmpA2 for hypermucoviscosity phenotype. The biofilm formation was significantly associated with ESBL production. OmpK36 group C isolates were more frequently detected in patients

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with community-acquired bloodstream infection. However, significant underlying diseases and prior use of carbapenem were highly prevalent in patients with OmpK36 non-C group isolates infection. ESBL production was apparently higher in non-C group but did not reach statistical significance.

Conclusion: Our results suggest that the OmpK36 group C *K.pneumoniae* is more associated with community-acquired infection with a lower frequency of underlying illness, but with significantly more virulence in bloodstream infection. This would give a remind that clinicians should be aware of such clinical impacts of the *ompK36* allele group.

Introduction

Klebsiella pneumoniae is a prominent cause of community-acquired and nosocomial infections worldwide.[1] The microorganism is also the second incidence as a cause of Gram-negative bacteremia after *Escherichia coli*.[2] Previous study showed that the OmpK36 is responsible for the exchange of extra- and intracellular substances, such as iron, nutrients and antibiotics, in *K. pneumoniae*.[3] The loss of OmpK36 porin could significantly affect the metabolic fitness of *K. pneumoniae*, and the slower growth rate would likely cause it to be much more easily eliminated in vivo by the immune system. In addition, alterations of OmpK36 in association with production of extended-spectrum beta-lactamases or cephalosporinases have been implicated in noncarbapenemase-mediated carbapenem-resistant phenogroups in *K. pneumoniae*.[4] Thus OmpK36 contributes to both the antimicrobial resistance and virulence of *K. pneumoniae*.

Previous studies have shown that *K. pneumoniae* OmpK36 plays a role in the inflammatory response during infections.[5] The loss of OmpK36 was shown to confer a lower virulence, decreased resistance to neutrophil phagocytosis and increased resistance to serum killing. Genetic polymorphism showed that *K. pneumoniae* isolates can be divided into four major ompK36 porin variants.[6] Moreover, among the four groups, group C was most likely associated with mucoid isolates, including all K1, K2, and K5 isolates, and most K57 isolates. Although highly virulent clones exhibiting loss of OmpK36 have been reported,[7] the association of OmpK36 porin variants with virulence and fitness in *K. pneumoniae* is yet to be completely understood. The purpose of this study was to investigate the microbiological characteristics and epidemiology of *K. pneumoniae* bloodstream isolates based on *ompK36* genotyping. We explored the relationship between OmpK36 porin variants and virulence features of *K. pneumoniae* bloodstream isolates with the corresponding patient demographics and host susceptibility factors.

Materials and methods

Patient information

A retrospective study was conducted at a tertiary university hospital with a 3500-bed capacity in central China from January 2015 to December 2015. Adult patients with at least one *K. pneumoniae* blood culture were retrospectively enrolled. Recovered *K. pneumoniae* strains were stored at −80 °C until analyzed. Clinical records were collected and reviewed including the following data: clinical and demographic data, underlying medical conditions, date of admission in the ICU, empirical antibiotics received and surgery performed within 7 days prior to positive culture of *K. pneumoniae*, use of central venous catheters, use of immunosuppressant, laboratory data (white blood cell [WBC] count and C-reactive protein [CRP]), and outcomes. The Acute Physiology and Chronic Health Evaluation (APACHE) II scores were calculated based on clinical data present during the twenty-four hours after the positive blood cultures were obtained. The primary outcome measure was all-cause 28-day mortality after the onset of *K. pneumoniae* bacteremia. The study was approved by the research ethics board at the first affiliated Hospital of Nanchang University.

Clinical *K. pneumoniae* isolates

A total of 80 consecutive cases of *K. pneumoniae* bloodstream isolates were collected. The microbiology laboratory performed identification and antimicrobial susceptibility of *K. pneumoniae* using the Vitek2 system (bioMérieux, Marcy-l'Etoile, France). Antimicrobial susceptibility was interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[8] Confirmation of the *K. pneumoniae* isolates was performed by 16S rRNA sequencing. ESBL production was confirmed by broth microdilution, according to CLSI guidelines. *E. coli* ATCC25922, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls for antimicrobial susceptibility testing.

Detection of virulence-associated features and genotyping

Hypermucoviscosity (HM) was identified by a positive string test as previously described.[9] The hypermucoviscosity phenotype was semi-quantitatively defined positive as a viscous string of >5 mm of the colony on blood agar plate.

Genomic DNA as templates, we undertook cps genotyping of K serotype-specific genes (including 6 liver abscess-

associated capsule serotypes K1, K2, K5, K20, K54 and K57), *iroB*, *kfuBC* and *allS* genes.[10,11] *K. pneumoniae* isolates were divided into groups A, B, C, and D by the PCR-based ompK36 typing method as described previously.[7] Plasmid DNA as templates, PCR was used to amplify the *rmpA*, *rmpA2* and aerobactin gene.[10,11] The PCR products were visualized by agarose gel electrophoresis and sequencing. The results were analyzed by BLAST program.

Detection of carbapenemase enzymes

A modified carbapenem inactivation method (mCIM) was performed to detect carbapenemases, as described previously.[8] The presence of plasmid mediated carbapenemase genes (KPC, NDM, VIM, IMP, OXA-48) were detected by PCR amplification followed by Sanger sequencing using the primers and conditions described elsewhere.[12]

Molecular typing

All isolates were subjected to MLST according to the protocol described by Liu et al.[13] Briefly, house-keeping genes, including *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*, were sequenced and compared to the MLST alleles profiles available at http://www.pasteur.fr/mlst (Genotyping of Pathogens and Public Health, Institut Pasteur, Paris, France).

Serum resistance assays

The susceptibility of the *K. pneumoniae* isolates to human serum was analyzed as described previously.[14] Briefly, 25 μL of bacterial suspension (about 2×10^6 CFU) was added to 75 μL of pooled normal human serum in microtiter plates. Viability was determined immediately and after 3 h of incubation at 37 °C by plating out serial dilutions on LB agar. The test was performed in triplicate and the number of recovered bacteria was determined and graded. Responses were graded as follows: "highly sensitive" (Grade 1 or 2), "intermediately sensitive" (Grade 3 or 4), or "resistant" (Grade 5 or 6).

Phagocytosis assay

Phagocytosis was measured using a standard assay as published and described.[15] The mean percentage of neutrophils that contained FITC-stained bacteria at 15 min in three repeated results was used as the phagocytosis rate.

Biofilm formation

Biofilm production was assessed using the microtitre plate assay, as described by the modified protocol of O'Toole and Kolter.[16] *K. pneumoniae* ATCC strains ATCC 700603 and ATCC 13883 were used as relatively high and low biofilm-forming isolates, respectively.

Mouse lethality experiment

Determination of the virulence of *K. pneumoniae* in mouse lethality tests and the median lethal dose (LD_{50}) was performed as described previously.[17] The degree of virulence was read as highly virulent for an LD_{50} of $<10^3$ CFU; moderate virulence for an LD_{50} of 10^4–10^5 CFU; low virulence for an LD_{50} of 10^6–10^7 CFU; and no virulence for an LD_{50} of $>10^8$ CFU.

Statistical analysis

Data are presented in mean ± standard deviation or median, range. Differences between patient data were analyzed by a Fisher's exact test. Survival data were analyzed with a log-rank test. Statistical tests were performed with GraphPad Prism 6 for Mac.

Results

Genotyping of clinical isolates

Among the 80 study isolates, OmpK36 groups A, B, C, and D were identified by PCR in 34 (42.5%), 6(7.5%), 26 (32.5%), and 12 (15%) isolates, respectively. Additionally, two novel OmpK36 groups were untypeable in one isolate each. The major differences between the allele groups were located between nucleotide position 500 and 1000 in the coding region (Fig. 1).

In general, the *allS*, *rmpA*, *rmpA2*, *iroB* and *aerobactin* genes were highly prevalent in OmpK36 group C isolates than ompK36 non-C group isolates (Table 1). Seventeen strains (21.25%) were identified as hypermucoviscosity *K. pneumoniae* (HMKP). Group C isolates have higher hypermucosicovity rates than non-C group isolates (50% vs. 7.4%). The common liver abscess-associated capsule serotypes were identified in 39 (48.75%) isolates by PCR methods, including capsule K1 (12 strains), K54 (2 strains), K20 (18 strains), 3 strains for K57 and K2 each, and one K5 respectively. These K1/K2 serotypes were highly prevalent in ompK36 group C isolates than in ompK36 non-C group isolates (15/26 vs. 0/54, $P = 0.0007$). However, the K20 serotype were highly prevalent in ompK36 non-C group isolates than in ompK36 group C isolates (16/54 vs. 2/26, $P = 0.0279$) (Table 1; Fig. 2A). Unexpectedly, eight K20 strains (44.4%) carried *rmpA2* gene and 10 strains (55.5%) carried *rmpA* gene, while only three strains showed the hypermucoviscosity phenotype. The prevalence of rmpA and rmpA2 of the K20 isolates might help to determine the low expression rates of rmpA and rmpA2 for hypermucoviscosity phenotype.

The OmpK36 group C isolates showed significantly lower antimicrobial resistant rates for almost all antimicrobials than ompK36 groups non-C isolates (Table 1). All but one group C isolate were susceptible to imipenem and meropenem. This group C strain was a carbapenemase-producing strain which was KPC positive. In contrast, fifteen OmpK36 non-C group isolates resistant to imipenem and meropenem were mCIM positive. Among them, fourteen strains were KPC positive (Table 1), and the KPC production was highly prevalent in ompK36 non-C group isolates than in ompK36 group C isolates (14/54 vs. 1/26, $P = 0.0178$) (Table 1).

Subsequent in silico MLST analysis identified 25 sequence types (STs) among the 80 K. *pneumoniae* bloodstream

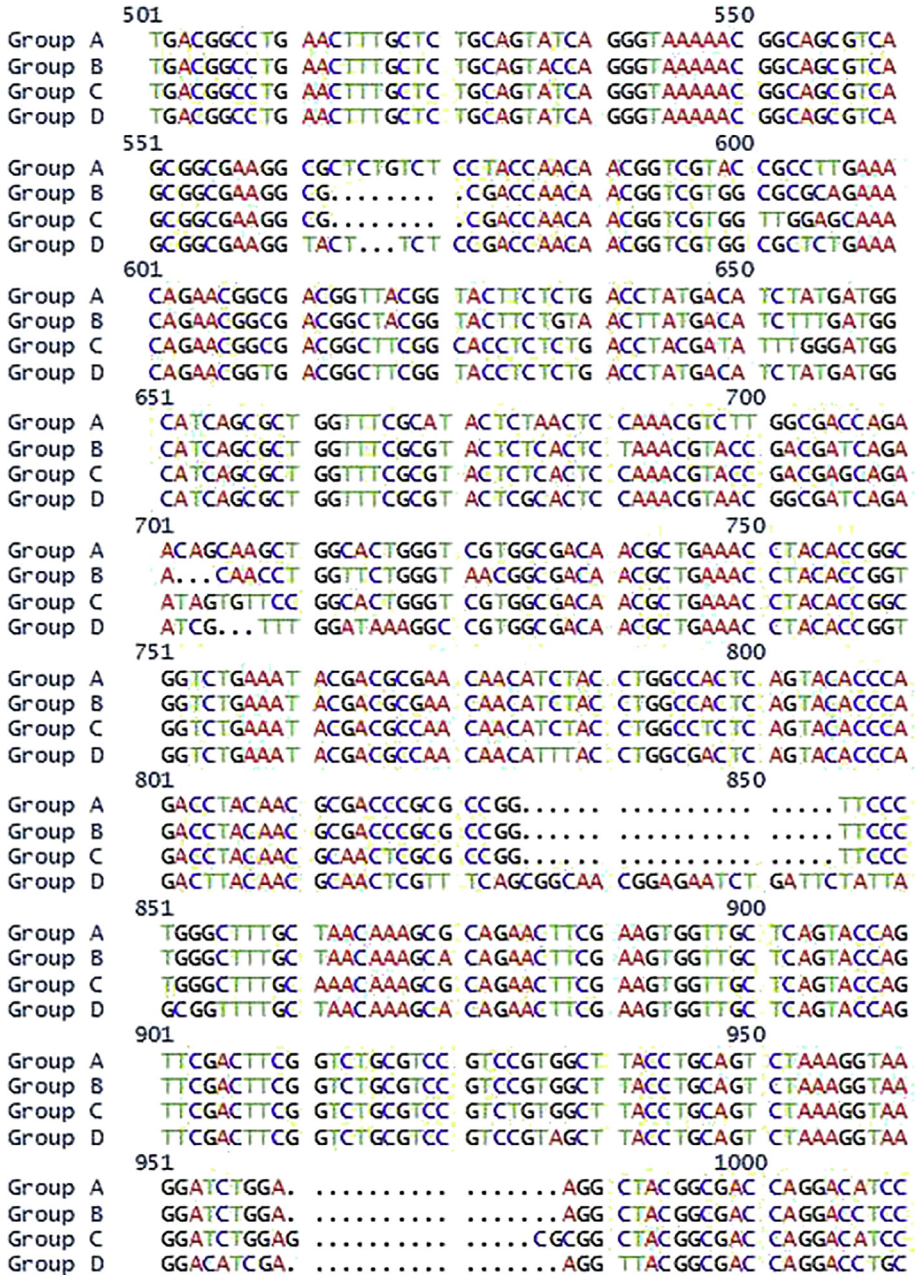

Figure 1. The genetic polymorphism of OmpK36 groups A, B, C, and D.

strains. Five different STs were confirmed in group C isolates. The most prevalent ST in group C strains was ST23 (12/26, 42.8%), followed by ST65 (6/26, 23.1%). All K1 *K. pneumoniae* belonged to ST23. In contrast, the most prevalent ST in non-C group isolates was ST11 (32/54, 59.3%) and they were all KPC positive *K. pneumoniae*, followed by ST147, ST25 and ST133.

Clinical characteristics

From January 2015 to December 2015, a total of 80 bloodstream infection patients were diagnosed with *K. pneumoniae*. The clinical characteristics of the patients with bloodstream infections are listed in Table 2. Episodes due to OmpK36 group C strains were less frequently nosocomial. Compared with OmpK36 non-C group, more patients with OmpK36 group C *K. pneumoniae* bacteremia had a significantly higher prevalence of community-acquired bloodstream infection (46.2% vs. 11.1%, $P = 0.0002$). A significantly higher number of patients presenting with no underlying diseases (46.2% vs. 7.4%, $P < 0.0001$) were infected by OmpK36 group C isolates. However, the use of carbapenem (11.5% vs. 46.3%, $P = 0.0023$) was most frequent in the patients with OmpK36 groups non-C *K. pneumoniae* bacteremia. The main comorbidities of patients with *K. pneumoniae* bacteremia were diabetes mellitus and hypertension. There was no statistically significant difference in severity of disease as expressed by APACHE II score and clinical outcomes between patients with OmpK36

Table 1 Microbiological characteristics and antimicrobial resistance of *Klebsiella pneumoniae*.

Variable	OmpK36 allele		*P* value
	Group C (N = 26)	Groups non-C (N = 54)	
Hypermucoviscosity	13 (50)	4 (7.4)	<0.0001*
Capsular serotype			
K1	12 (46.2)	0 (0)	<0.0001*
K2	3 (11.5)	0 (0)	0.0109*
K20	2 (7.7)	16 (29.6)	0.0279*
K54	1 (3.8)	1 (1.9)	0.5926
K57	2 (7.7)	1 (1.9)	0.1978
K5	1 (3.8)	0 (0)	0.1470
Untyped	5 (19.2)	36 (66.7)	<0.0001*
Virulence gene			
allS	5 (19.2)	2 (3.7)	0.0213*
rmpA2	16 (61.5)	10 (18.5)	0.0001*
rmpA	17 (65.4)	13 (24.1)	0.0004*
Aerobactin	17 (65.4)	13 (24.1)	0.0004*
iroB	5 (19.2)	0 (0)	0.0213*
mrkD	26 (100)	54 (100)	NA
kfuBC	12 (46.2)	18 (33.3)	0.2673
Antimicrobial susceptibility			
Cefoxitin	8 (30.8)	28 (51.9)	0.0758
Cefotaxime	7 (26.9)	24 (44.4)	0.1319
Ceftazidime	7 (26.9)	26 (48.1)	0.0709
Cefepime	5 (19.2)	22 (40.7)	0.0567
Gentamicin	8 (30.8)	28 (51.9)	0.0758
Imipenem	1 (3.8)	15 (27.8)	0.0122*
Meropenem	1 (3.8)	14 (25.9)	0.0178*
Amikacin	1 (3.8)	11 (20.3)	0.0525
Ciprofloxacin	10 (38.5)	25 (46.3)	0.5082
ESBLs production	7 (26.9)	26 (48.1)	0.0709
KPC production	1 (3.8)	14 (25.9)	0.0178*
Biofilms formation	0.8923 ± 0.4360	0.7492 ± 0.5205	0.1348

Values are presented as no. (%) of *Klebsiella pneumoniae*.
ESBL extended spectrum β-lactamase, NA not applicable.
*$P < 0.05$ compared with ompK36 groups non-C *K. pneumoniae* isolates.

group C isolates and OmpK36 groups non-C isolates. Seventeen (65.4%) of the 26 group C isolates were responsible for PLA-associated bacteremias, and the rate was significantly higher than the rates in non-C group (7.4%). There were no significant differences in bacteremias related to pneumonia, peritonitis, urinary tract infections, and other infections among the four groups (data not shown).

Serum killing resistance, biofilm formation and phagocytosis

In general, the distribution of isolates in the grading of serum resistance were 30 strains in grades 5 and 6; grade 4 and 3 (27 strains); and grade 2 and 1 (23 strains) (Fig. 2A). The vast majority (88.5%, 23/26) of OmpK36 group C isolates were classified as either highly serum-resistant or serum-resistant, compared to only 62.9% (34/54) of OmpK36 non-C group isolates ($P = 0.0195$; Fig. 2B). However, the 30 isolates with high serum resistance (grades 5–6) were still significantly associated with K1/K2 serotypes (n = 8, $P < 0.0001$) and HM phenotype (n = 10, $P = 0.0174$), in comparison to isolates with lower grade serum resistance (grades 1–4) (Fig. 2C).

The phagocytosis rates of these bacteremia strains were highly variable, where the mean 15-minute phagocytic rates (±SD) for OmpK36 non-C group isolates and OmpK36 group C isolates were 63.20 ± 15.60% and 30.40 ± 10.50%, respectively (Fig. 4). Overall, capsular serotype K1/K2, both ST23 and ST65 strains, were relatively more resistant to phagocytosis (79.21 ± 7.54% and 66.76 ± 12.15%, respectively) than the other strains (data not shown).

The ability of *K. pneumoniae* bloodstream isolates to form biofilms in this crystal violet-based assay varied considerably, but most strains form relatively high-diversity biofilms (OD_{570} range 0.0578–1.8223) in this system. No statistically significant variation was detected between the OD values of the OmpK36 group C isolates and those of the

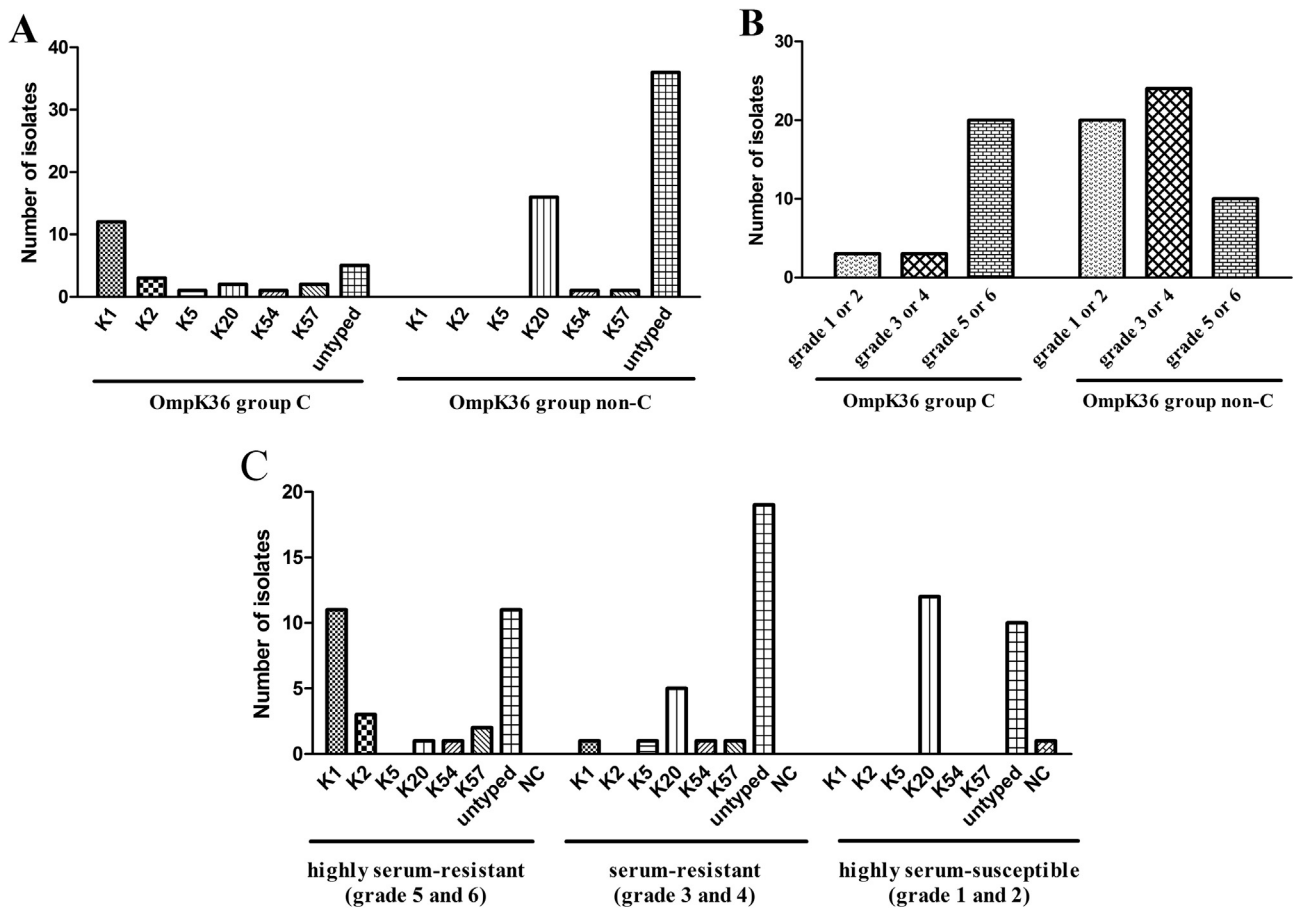

Figure 2. (A)The proportions of capsule types among the OmpK36 group C *K.pneumoniae* isolates and non-C group isolates. (B) OmpK36 group C *K.pneumoniae* isolates were more resistant to serum exposure than OmpK36 non-C group isolates. Comparison of prevalence of serum-resistant (grade 5 or 6) population between OmpK36 group C *K.pneumoniae* isolates and OmpK36 non-C group isolates (76.9% vs 18.5%; $P < 0.0001$). (C) Serum resistance level of isolates from the different capsule types. The negative control (NC), *E. coli* OP50, was highly-serum susceptible.

OmpK36 groups non-C isolates (OD_{570} values (mean $\pm$ SD) 0.8923 ± 0.4360 vs. 0.7492 ± 0.5205, $P = 0.1348$) (Fig. 3A; Table 1). However, the biofilm formation of *K. pneumoniae* isolates was significantly associated the strains with ESBL production, regardless of OmpK36 genotype (Fig. 3).

Mouse lethality experiments

$LD_{50} < 10^6$ (high virulence) was found in 18 of 26 OmpK36 group C isolates, but in 10 of 54 OmpK36 non-C group isolates ($P < 0.0001$). The prevalence of virulence factors were significantly higher in isolates with $LD_{50} < 10^6$ (high virulence) than those with low virulence ($LD_{50} > 10^6$), including K1/K2 serotypes (15/28 vs. 0/52, $P < 0.0001$) and HM phenotype (10/28 vs. 7/52, $P = 0.0203$) (data not shown).

Discussion

Outer membrane protein is responsible for the exchange of nutrients and toxic metabolites.[18] The accumulation of internal toxic metabolites or blocking the entry of nutrients may reduce the virulence of bacteria.[5] Porins are represented in large amounts in outer membrane and form non-specific diffusion channels.[19] Alteration or loss of OmpK36 porin is one of the important mechanisms that contributes to carbapenem resistance in *K. pneumoniae*.[20] It is more likely that sequence heterogeneity of OmpK36 may be due to the selective pressures created by both host immunity and antimicrobial use. The goal of the present study was to evaluate the impact of OmpK36 porin variants on the clinical characteristic and virulence of *K. pneumoniae*.

Our study reports the association between *ompK36* allele groups of *K. pneumoniae* and demographics and microbiological characteristics of *K. pneumoniae* bacteraemia patients. We found that the most virulent and widespread capsule type K1/K2 in the group C isolates were significantly higher frequent than the non-C group (A, B, D and nontypeable groups). One plausible explanation for this finding could be the association of the major high-risk clones and virulent STs with specific *ompK36* allele groups.[7] Group C isolates were more likely to be community acquired than the isolates of the other groups, while groups non-C isolates tended to be nosocomially acquired. The OmpK36 group C *K. pneumoniae* isolates were typed into

Table 2 Baseline characteristics of patients with *Klebsiella pneumoniae* bloodstream infection.

Characteristic	Patients with OmpK36 group C *K.pneumoniae* bacteremia (N = 26)	Patients with OmpK36 groups non-C *K.pneumoniae* bacteremia (N = 54)	P value
Age, years	51.0 (25–85)	55.9 (17–89)	0.8173
Male sex	14 (53.85)	29 (53.7)	0.9905
Community-acquired	12 (46.2)	5 (11.1)	0.0002*
APACHE II score >15	14 (53.8)	26 (48.1)	0.6331
Use of immunosuppressant	2 (7.7)	4 (7.4)	0.9639
Insertion of central venous catheter	9 (34.6)	20 (37.0)	0.8329
Infection occurred in ICU	3 (11.5)	17 (31.5)	0.0537
C-reactive protein (median, Q1-Q3)	39.9 (37.6–67.1)	31.1 (14.2–90.5)	0.6060
White blood cell count (median, Q1-Q3)	13,200 (3700–17,800)	9900 (5900–13,000)	0.7760
Underlying disease			
No underlying disease	12 (46.2)	4 (7.4)	<0.0001*
Diabetes mellitus	6 (23.1)	6 (11.1)	0.1604
Hypertension	6 (23.1)	9 (16.67)	0.4914
Chronic pulmonary disease	0 (0)	0 (0)	NA
Chronic liver disease	1 (3.8)	0 (0)	0.1470
Surgery within 7 days prior to *K. pneumonia* isolated	4 (15.4)	12 (22.2)	0.4739
Empirical antibiotics received			
Any antibiotic	22 (84.6)	43 (79.6)	0.5926
Penicillin	1 (3.8)	0 (0)	0.1470
Cephalosporin	8 (20.8)	9 (16.67)	0.1487
Carbapenem	3 (11.5)	25 (46.3)	0.0023*
β-Lactam/β-lactamase inhibitor			
Combinations	14 (53.8)	28 (51.9)	0.8671
Aminoglycoside	1 (3.8)	0 (0)	0.1470
Tetracycline	1 (3.8)	0 (0)	0.1470
Sulfonamide	0 (0)	0 (0)	NA
Mortality	13 (50)	29 (53.7)	0.7560

Values are presented as no. (%) of patients.
NA not applicable.
*$P < 0.05$ compared with patients infected by ompK36 groups non-C *K. pneumoniae* isolates.

five different groups by MLST. ST23 predominated and contained twelve isolates indicating that the OmpK36 group C *K. pneumoniae* isolates were spread by clonal dissemination. Moreover, a total of 20 currently available STs were identified in the OmpK36 group non-C *K. pneumoniae* isolates, with ST11 and ST147 as the most common STs.

It has been reported that the OmpK36-deficient strain is more resistant to serum killing.[21] Moreover, the *K. pneumoniae* OmpK36 can also activate the classical complement pathway via antibody independent binding to C1q.[22] In this study, a significant increase in resistance to serum killing was observed for OmpK36 group C isolates. Therefore, the

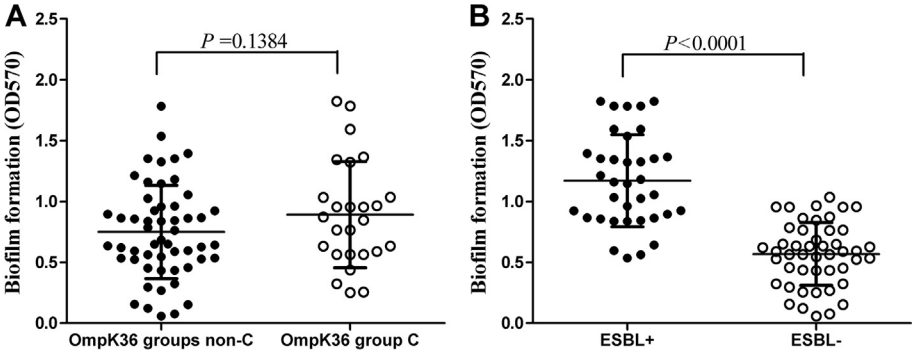

Figure 3. (A) Comparison of the biofilm formation between OmpK36 group C *K.pneumoniae* isolates and OmpK36 non-C group isolates. (B) Comparison of the biofilm formation between ESBL-positive and ESBL-negative *K.pneumoniae* isolates. Biofilms (expressed in OD570 values) were prepared from each isolate using the standard crystal violet staining method.

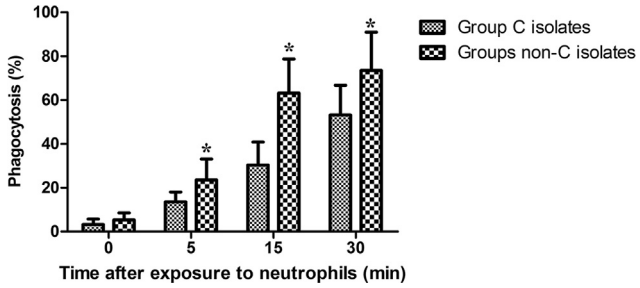

Figure 4. Neutrophils phagocytosis assays among OmpK36 group C isolates and non-C group isolates. Data are presented as mean phagocytic rate ± standard deviation (SD) at 0 min, 5 min, 15 min, and 30 min. The phagocytic rates were highly variable with mean 15-minute phagocytic rates (±standard deviation) for OmpK36 group C isolates and non-C group isolates.

characteristic of resistance to serum killing likely contribute to the success of OmpK36 group C isolates. Whether this effect is due to the C1q binding sites being activated by the group C OmpK36 requires further study. Previous studies have shown that knockout of ompK36 can modify the surface structure of *K. pneumoniae* and this modification may alter the receptor binding of phagocytes leading to increased susceptibility to phagocytosis.[3] Mounting evidence indicates that *Klebsiella* OmpK36 also contribute to phagocytosis resistance in *K. pneumoniae*. Our results revealed that OmpK36 group C isolates were more resistant to phagocytosis than group non-C isolates (Fig. 4).

Biofilm has been studied in hypervirulent *K. pneumoniae* strains with K1 or K2 serotypes.[23] Biofilm formation was variable among all *K. pneumoniae* strains but no correlation with C-type was documented, although 15 of 26 ompK36 group C *K. pneumoniae* isolates was identified as K1/K2 serotypes in this study. However, it should be noted that these results are based on our in vitro model and might change in vivo.[24] A dramatic increase in antibiotic resistance in biofilm state has also been described previously.[25,26] Biofilm promotes plasmid stability, and acquiring plasmids harbouring ESBL encoding genes is the predominant mechanism associated with the increase of ESBL-producing *K. pneumoniae*. Therefore, *K. pneumoniae* has high tendency to form biofilm which is strongly associated with the ESBL production.[27] In the current study, biofilm was highly prevalent in ESBL producers. More importantly, development of strong biofilm is much more significant in ESBL producers compared to the non-ESBLs ($P < 0.0001$, Fig. 3B).

The mice lethality study showed that OmpK36 group C *K. pneumoniae* isolates were more virulent than group non-C isolates. Furthermore, our genotypic data revealed that OmpK36 group C *K. pneumoniae* isolates were more commonly associated with acquired virulence genes than group non-C isolates, supporting the hypothesis that OmpK36 variants may play a significant role in the virulence of *K. pneumoniae*.[28] There were two limitations associated with this study. First, the current study was a single center, retrospective analysis with a limited sample size. Second, it would be possible that ompK36 allele group C indicates wild-type strain of *K. pneumoniae* that are hypervirulent whereas non-C groups refer to classic type *K. pneumoniae* that are traditionally less virulent. Further large-scale epidemiological study is warranted.

As a whole, OmpK36 group C *K. pneumoniae* isolates were assumed to be more virulent than groups non-C isolates. First of all, OmpK36 group C *K. pneumoniae* isolates showed a higher rate of resistance against human serum and phagocytosis than non-C group isolates. Second, the hypermucoviscosity phenotype and virulent K1/K2 serotypes were more frequently found in OmpK36 group C *K. pneumoniae* isolates. Finally, we also provided evidence that the OmpK36 group C *K. pneumoniae* isolates were significantly higher virulent than OmpK36 non-C group isolates by the mouse lethality. The correlation between OmpK36 variants and the virulence features of *K. pneumoniae* may provide valuable information of management of *K. pneumoniae* bloodstream infections. Our work paves the way for future investigation into the dual role of OmpK36 variants in driving the epidemiology of *K. pneumoni*ae.

Conflicts of interest

All authors declare no conflict of interest.

Acknowledgments

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