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ORIGINAL ARTICLE

Regulator of the mucoid phenotype A gene increases the virulent ability of extended-spectrum beta-lactamase-producing serotype non-K1/K2 *Klebsiella pneumonia*

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KEYWORDS
β-lactamase-producing *Klebsiella pneumonia*;
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regulator of mucoid phenotype A

Background: To determine whether the presence of a capsule regulator gene [i.e., regulator of mucoid phenotype A (*rmpA*) gene] contributes to virulence on extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) with serotype non-K1/K2 strains.
Methods: Twenty-eight ESBL-KP and non-ESBL-KP isolates were collected from the Tri-Service General Hospital (Taipei, Taiwan). The impact of the virulent *rmpA* gene in different capsular polysaccharide serotypes on ESBL-KP and non-ESBL-KP isolates was studied by a neutrophil phagocytosis reaction, a serum bactericidal assay, and an animal survival model.
Results: Resistance to broad spectrum antibiotics was more prevalent in ESBL-KP strains than in non-ESBL-KP strains ($p < 0.01$). The ESBL-KP strains had different molecular patterns from non-ESBL-KP strains, based on pulsed-field gel electrophoresis. The frequency of serum-resistant isolates was the highest among ESBL-KP strains with *rmpA* (i.e., *rmpA*$^+$) [71.4% (5/7)] than among of non-ESBL-KP *rmpA*$^+$ strains [42.8% (6/14)], ESBL-KP strains without *rmpA* (*rmpA*$^-$) [33.3% (7/21)], and non-ESBL-KP *rmpA*$^-$ strains [14.2% (2/14)]. The most significant increase in neutrophil resistance occurred in the ESBL-KP *rmpA*$^+$ strains in comparison to

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the non-ESBL-KP *rmpA*$^+$, ESBL-KP *rmpA*$^-$, and non-ESBL-KP *rmpA*$^-$ strains ($p < 0.01$). The results of the animal survival model were compatible with the neutrophil phagocytosis reaction and serum bactericidal assay.
Conclusion: We conclude that the pathogenic potential is greater in *rmpA*$^+$ ESBL-KP strains than in *rmpA*$^-$ ESBL-KP and non-ESBL-KP strains.

Introduction

In recent decades, the incidence of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) in patients with nosocomial infections has increased.[1–5] The coexistence with other resistant mechanisms has made this opportunistic pathogen more difficult to treat because effective antibiotic options are limited.[6–8] Carbapenem is not yet very effective for ESBL-KP, although in recent years increasing reports of carbapenem resistance have been of great concern.[8–10] Furthermore, ESBL-KP is reportedly more virulent than non-ESBL-KP. Increased serum resistance and impairment of the respiratory burst within neutrophils have been documented in ESBL-KP and non-ESBL-KP strains.[11,12]

Different virulence factors such as lipopolysaccharides (O-antigen), capsular polysaccharides (K-antigen), fimbrae, and siderophores have been reported in *K. pneumoniae*.[13] In addition, different genes related to virulence have also been documented. Serotypes K1 and K2 are the most virulent types of *K. pneumoniae* in community-acquired infections and in animal studies.[14–16] The antiphagocytic properties of serotypes K1 and K2 may contribute to the high virulence[17]; however, serotypes K1 and K2 of *K. pneumoniae* are rarely encountered in nosocomial infections.[18] The virulence for serotypes non-K1/K2 of *K. pneumoniae* has not been reported in nosocomial infections involving ESBL-KP. In addition to capsular serotype K antigens, a capsule regulator gene [i.e., regulator of mucoid phenotype (*rmpA*)] reportedly contributes to resistance in neutrophil phagocytosis.[16,19] Taken together, additional studies are needed concerning whether *rmpA* in strains of ESBL-KP with non-K1/K2 contributes to high virulence in non-ESBL-KP. In the present study, we collected 28 nosocomial non-ESBL-KP isolates and ESBL-KP isolates and studied their virulence, based on the presence of *rmpA* in *in vitro* and *in vivo* virulence assays.

Materials and methods

Collection of ESBL-KP and non-ESBL-KP isolates

K. pneumoniae isolates were collected between January 2008 and June 2009 at the Tri-Service General Hospital (Taipei, Taiwan) from patients with nosocomial bacteremia. The strains of *K. pneumoniae* bacteremia were collected from nosocomial bacteremia with ESBL-KP strains and non-ESBL-KP strains. The criterion for a nosocomial bacteremia was that the strain was isolated from patients with bacteremia after 48 hours of admission to the hospital. The ESBL-KP producers were reconfirmed using the disc diffusion method. The ESBL discs were impregnated with cefotaxime/clavulanic acid (30 g/10 g) and cefotaxime (30 g), and ceftazidime/clavulanic acid (30 g/10 g) and ceftazidime (30 g; Becton Dickinson, Franklin Lakes, NJ, USA). An increase of 5 mm or greater in the zone diameter for clavulanic acid-supplemented discs, compared to plain discs, indicated an ESBL producer.[20] In this study, 56 strains (28 ESBL-KP and 28 non-ESBL-KP strains) were selected randomly. Serotyping was assessed by the capsular swelling technique and counter current immunoelectrophoresis.[15] The mucoviscosity was assessed using a string test, as previously reported.[15]

The determined minimal inhibitory concentrations of different strains of *K. pneumonia*

Fifty-six different strains of *K. pneumoniae* were obtained from patients with bacteremia. The minimal inhibitory concentrations (MIC) of antimicrobial agents were determined by the E-test in accordance with the recommendations from the Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA).[21] We determined antimicrobial susceptibility to ampicillin, amoxicillin/clavulanate, piperacillin, aztrenam, cefazolin, ceftazidime, cefotaxime, cefepime, gentamicin, amikacin, ciprofloxacin, and imipenem, based on recommendations of the CLSI.[22] The breakpoint of moxalactam was used for flomoxef.[22]

Pulsed-field gel electrophoresis analysis

Total DNA was prepared and pulsed-field gel electrophoresis (PFGE) was performed, as previously described.[23] The restriction enzyme *Xba*I (New England Biolabs, Beverly, MA, USA) was used at the manufacturer's suggested temperature. Restriction fragments were separated by PFGE in 1% agarose gel (Bio-Rad, Hercules, CA, USA) in 0.5 × Tris/Borate/EDTA (TBE) buffer (45 mM Tris, 45 mM boric acid, and 1.0 mM EDTA; pH 8.0) for 22 hours at 200 V at a temperature of 14°C with ramped times of 2—40 seconds using the Bio-Rad CHEF MAPPER apparatus (Bio-Rad Laboratories, Richmond, CA, USA). The gels were then stained with ethidium bromide and photographed under UV light. The resulting genomic DNA profiles or "fingerprints" were interpreted in accordance with established guidelines.[24]

Detection of ESBL-KP types and *rmpA* using polymerase chain reaction

Polymerase chain reaction (PCR) was used to target specific genes for the ESBL-KP types and *rmpA* gene. For detecting the *rmpA* gene, a primer was designed using previously published sequences.[16,19] An overnight-cultured bacterial colony was added to 300 μL of water and boiled for 15 minutes to release the DNA template. For detecting the ESBL-KP genes, conserved primers sets of SHV-type, TEM-type, and CTX-M-type β-lactamases were selected (Table 1). The PCR products were sequenced to determine the ESBL-KP types.[25–28] A chromosomal 16S rDNA gene was the internal positive control for each PCR reaction with primers designed as follows: forward, 5′-ATCTGGTGGACTACTCGC-3′; and reverse, 5′-GCCTCATTCAGTTCCGTT-3′.

The reaction mixture was maintained at 95°C for 5 minutes, followed by 40 temperature cycles of 95°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes, and 72°C for 7 minutes.[16,19,25–28]

Phagocytosis assay

Neutrophil isolation from healthy volunteers and bacteria labeling with fluorescein isothiocyanate (FITC) were performed, as previously described.[17] A mixture consisting of labeled bacteria, a neutrophil suspension, pooled normal human serum, and phosphate buffered saline (PBS; pH, 7.4) was incubated for reaction of 0 minutes and 10 minutes in a shaking water bath at 37°C. After centrifugation, the supernatant was removed and the cell pellet was resuspended in an ice-cold PBS solution. Ethidium bromide solution was added. Fluorescein isothiocyanate fluorescence was detected using the FACScan scanner (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Using a logarithmic amplifier, fluorescence distribution data were displayed as single histograms for FL1-H. The mean percentage of neutrophils that contained FITC-stained bacteria at 15 minutes in six repeated results was used as the phagocytosis rate.

Table 1 Specific primers used for amplification of the target alleles of different *rmpA* genes and ESBL genes of *K. pneumoniae*

Target gene (group)	Primer	Size of product (bp)
RmpA		583
Forward	5′-CAGTTAACTGGACTACCTCTG-3′	
Reverse	5′-GAAAGAGTGCTTTCACCCCCT-3′	
SHV		931
Forward	5′-GGGTTATTCTTATTTGTCGC-3′	
Reverse	5′-TTAGCGTTGCCAGTGCTC-3′	
TEM		1079
Forward	5′-ATAAAATTCTTGAAGACGAAA-3′	
Reverse	5′-ATAAAATTCTTGAAGACGAAA-3′	
CTX-M		550
Forward	5′-CGCTTTGCGATGTGCAG-3′	
Reverse	5′-ACCGCGATATCGTTGGT-3′	

rmpA = regulator of mucoid phenotype A.

Serum killing assay

Serum bactericidal activity was measured using a modification of the method by Hughes and Podschun.[29,30] Bacteria grown in nutrient broth were collected during the early logarithmic phase. The viable bacteria concentration was adjusted to 1×10^6 colony-forming units (CFU)/mL and reacted with 75% pooled human sera. A strain was considered "serum-resistant" or "serum-sensitive" if the grading was the same in all experiments. Each isolate was classified as "highly sensitive" (Grade 1 or 2), "intermediately sensitive" (Grade 3 or 4), or "resistant" (Grade 5 or 6). Each strain was tested at least three times.

Mice lethality study

Pathogen-free male BALB/c mice (age, 6–8 weeks old; weight, 20–25 g) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). All experiments involving mice were approved by the Committee on Institutional Animal Care and Use of the Tri-Service General Hospital and National Defense Medical Center (Taipei, Taiwan). Mice were injected intraperitoneally with the designated strains. The inoculum contained 10^6 CFU and 10^7 CFU. Mouse mortality was assessed for up to 14 days. Each experiment with six mice of each strain was performed three times and repeated twice.

Statistical analysis

The Student *t* test was used for statistical analysis. Data are expressed as the mean ± standard deviation (SD). Univariate analyses were performed to compare the differences between ESBL-KP and non-ESBL-KP in the adjusted serum resistance and neutrophil killing. Data are expressed as the least-squares mean ± standard error of the mean. The log-rank test was used for statistical analyses of survival curves. All statistical tests were two-sided. A p value <0.05 was considered statistically significant.

Results

Serotypes of *Klebsiella pneumonia*

The serotypes for non-K1/K2 non-ESBL-KP were K16 ($n = 3$), K20 ($n = 1$), K22 ($n = 2$), K28 ($n = 1$), K30 ($n = 1$), K38 ($n = 1$), K54 ($n = 2$), K55 ($n = 4$), and non-typed serotypes ($n = 13$). The serotypes for non-K1/K2 ESBL-KP were K6 ($n = 1$), K7 ($n = 1$), K12 ($n = 1$), K15 ($n = 1$), K18 ($n = 1$), K27 ($n = 1$), K35 ($n = 1$), K46 ($n = 1$), K54 ($n = 1$), K55 ($n = 1$), and non-typed serotypes ($n = 18$). None of the enrolled strains exhibited mucovisicosity. Fifty percent (14/28) of the non-K1/K2 non-ESBL-KP strains had the *rmpA*$^+$ gene. Twenty-five percent (7/28) of the non-K1/K2 ESBL-KP strains had the *rmpA*$^+$ gene.

Antimicrobial susceptibility of clinical isolates

Twenty-eight non-K1/K2 ESBL-KP strains and 28 non-ESBL-KP strains were enrolled. They were tested with penicillin,

aztreonam, cephalosporin, aminoglycosides, quinolone, and carbapenem, based on CLSI criteria. The results showed 100% of the strains had resistance to ampicillin, piperacillin, cefazolin, cefotaxime, cefepime, and gentamicin. In the non-K1/K2 ESBL-KP group, the resistance rate ranged 37.5—68.7% for amoxillin/clavanate, aztreonem, ceftazidime, amikacin, and ciprofloxacin. In the non-ESBL-KP group, 100% of the strains showed resistance to ampicillin and 100% of the strains were susceptible to the other antibiotics (Table 2).

Types of ESBL-KP and PFGE

Fifty-six strains were tested by an ESBL confirmatory assay and 28 strains were confirmed as ESBL-KP. Twenty-eight isolates were enrolled and studied for the ESBL-KP types. Polymerase chain reaction amplification and sequencing of the PCR products revealed the CTX-M type [100% (28/28)] existed concomitantly with the SHV-5 type [17.8% (5/28)] and the SHV-12 type [11% (2/28)]. Three CTX-M types were identified, among which CTX-M-3 was the most common ESBL-KP type [92.8% (26/28) of enrolled isolates], followed by CTX-M-14 and CTX-M-15 [3.5% (1/28) for each]. Molecular typing of ESBL-KP isolates (Fig. 1A) and non-ESBL-KP isolates (Fig. 1B) confirmed that all strains collected in this study were unrelated clonally.

Serum resistance in relation to ESBL-KP strains

The results were reproducible in all three experiments for each strain. The percentage of serum resistance was significantly higher among the ESBL-KP strains than among the non-ESBL-KP strains [42.9% (12/28) vs. 28.6% (8/28), respectively; $p = 0.26$]. The percentage of serum resistance was significantly higher among the *rmpA*$^+$ strains than among the *rmpA*$^-$ strains [52.4% (11/21) vs. 25.7% (9/35), respectively; $p = 0.043$]. In particular, *rmpA*$^+$ ESBL-KP strains [71.4% (5/7)] had the highest serum resistance rate, compared to the *rmpA*$^+$ non-ESBL-KP strains [42.8% (6/14)], the *rmpA*$^-$ ESBL-KP strains [33.3% (7/21)], and the *rmpA*$^-$ non-ESBL-KP strains [14.2% (2/14)].

Effect of *rmpA* on neutrophil phagocytosis of ESBL-KP isolates

Neutrophil phagocytosis of ESBL-KP isolates was significantly decreased, compared to that of non-ESBL-KP isolates ($68.45 \pm 1.63\%$ and $75.92 \pm 1.37\%$, respectively; $p = 0.001$; Fig. 2A). Neutrophil phagocytosis of *rmpA*$^+$ *K. pneumoniae* was significantly reduced ($68.81 \pm 1.95\%$), compared to the *rmpA*$^-$ strains ($75.95 \pm 0.79\%$; $p = 0.002$; Fig. 2B). The effect of rmpA gene on phagocytosis was further ascertained. The phagocytosis rate of the four groups (i.e., *rmpA*$^+$ ESBL-KP strains, *rmpA*$^-$ ESBP-KP strains, *rmpA*$^+$ non-ESBL-KP strains, and *rmpA*$^-$ non-ESBP-KP strains) are displayed in Fig. 2C. *RmpA*$^-$ non-ESBL-KP isolates were all highly susceptible to neutrophil phagocytosis at 30 minutes with a phagocytic uptake rate of $80.67 \pm 3.63\%$. No significant difference was observed in the phagocytic rate of *rmpA*$^-$ ESBL-KP isolates and *rmpA*$^+$ non-ESBL-KP isolates ($71.16 \pm 3.4\%$ vs. $73.35 \pm 1.65\%$, respectively; $p = 0.87$; Fig. 2C). For ESBL isolates, the phagocytosis rate was lower in the strains with *rmpA*$^+$ than in the strains with *rmpA*$^-$ ($54.14 \pm 1.85\%$ vs. $73.35 \pm 1.65\%$, respectively; $p = 0.007$). For the non-ESBL-KP isolates, the phagocytosis rate was lower in the strains with *rmpA*$^+$ than in the strains with *rmpA*$^-$ ($71.16 \pm 3.4\%$ vs. $80.67 \pm 3.63\%$, respectively; $p < 0.001$).

Mice lethality study

To further explore the role of the *rmpA* gene in ESBL-KP pathogenicity, we examined the different strains of the *rmpA*$^{+/-}$ gene *K. pneumoniae* in BALB/c mice. The study

Table 2 The minimal inhibitory concentrations of different antibiotics in ESBL strains and non-ESBL-producing *K. pneumoniae* strains

	ESBL-producing *K. pneumoniae* ($n = 28$)				Non-ESBL-producing *K. pneumoniae* ($n = 28$)			
	MIC range (mg/L)	S (%)	I (%)	R (%)	MIC range (mg/L)	S (%)	I (%)	R (%)
Ampicillin	>16	0	0	100	>16	0	0	100
Amoxicillin/clavulanate	8 ~ >16	12.5	25	62.5	<4	100	0	0
Piperacillin	>64	0	0	100	<2	100	0	0
Aztreonam	4 ~>16	31.25	0	68.75	<4	100	0	0
Cefazolin	>16	0	0	100	<2	100	0	0
Ceftazidime	2~>16	12.5	25	62.5	<2	100	0	0
Cefotaxime	>16	0	0	100	<2	100	0	0
Flomoxef	<2 ~ >16	75	12.5	12.5	<2	100	0	0
Cefepime	>16	0	0	100	<0.5	100	0	0
Gentamicin	>8	0	0	100	<0.16	100	0	0
Amikacin	<2 ~ >32	56.25	6.25	37.5	<2	100	0	0
Ciprofloxacin	<0.03 ~ >2	50	6.25	43.75	<0.03	100	0	0
Imipenem	<0.25 ~ >8	93.75	0	6.25	<0.25	100	0	0

Minimal inhibitory concentration (MIC) interpretation criteria for antimicrobial agents with/without Clinical and Laboratory Standards Institute criteria were determined, based on the description in References 21 and 22.
ESBL = extended-spectrum beta-lactamase; *K. pneumoniae* = *Klebsiella pneumoniae*; MIC = minimal inhibitory concentration; S = susceptibility; I = intermediates; R = resistance.

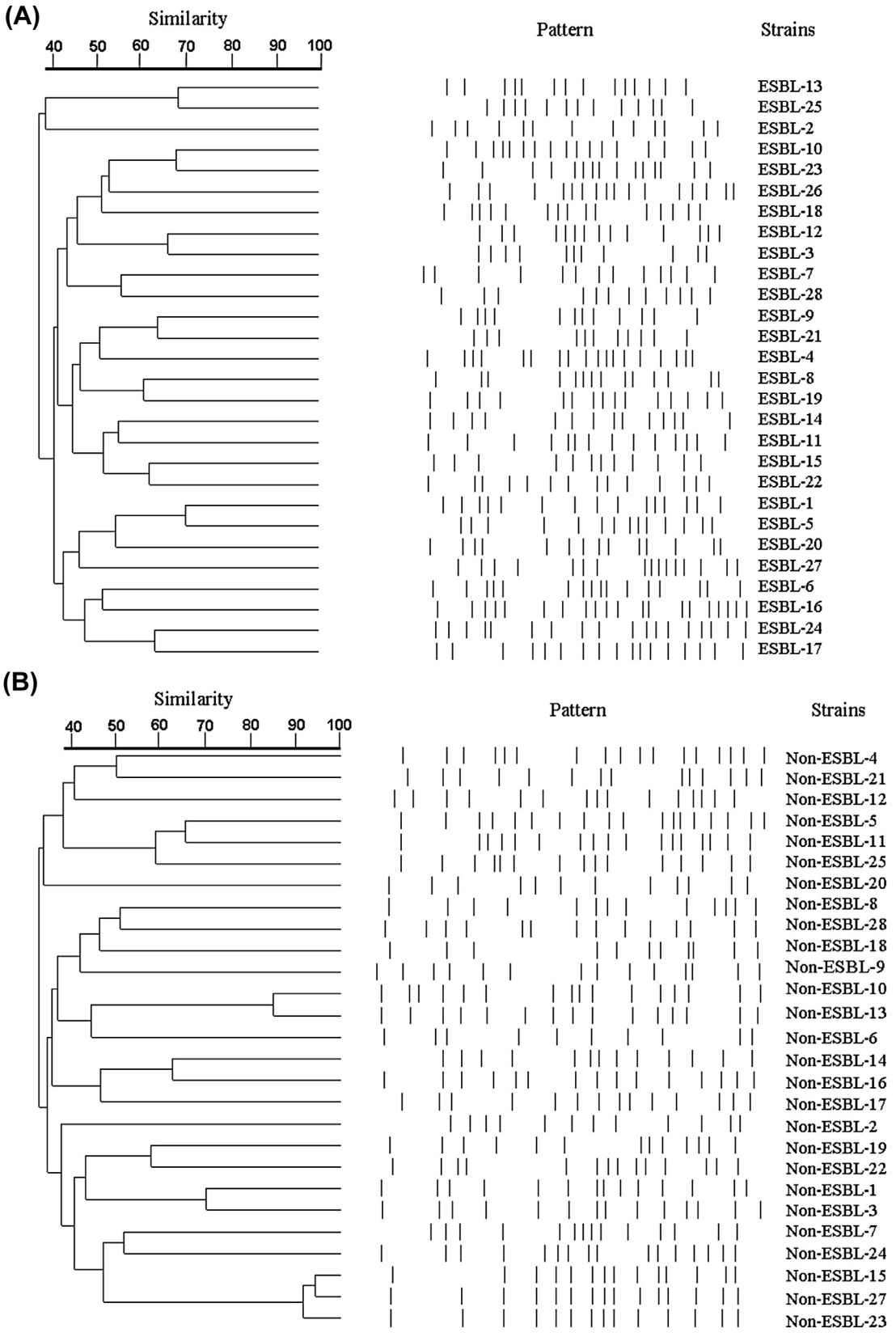

Figure 1. (A) Molecular typing of 28 extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) by pulsed-field gel electrophoresis (PFGE). (B) Molecular typing of 28 non-ESBL-KP by PFGE. Isolates with $\geq$80% similarity (i.e., $<$3-band difference) are defined as clonal type strains. A different high genetic polymorphism was observed in each group.

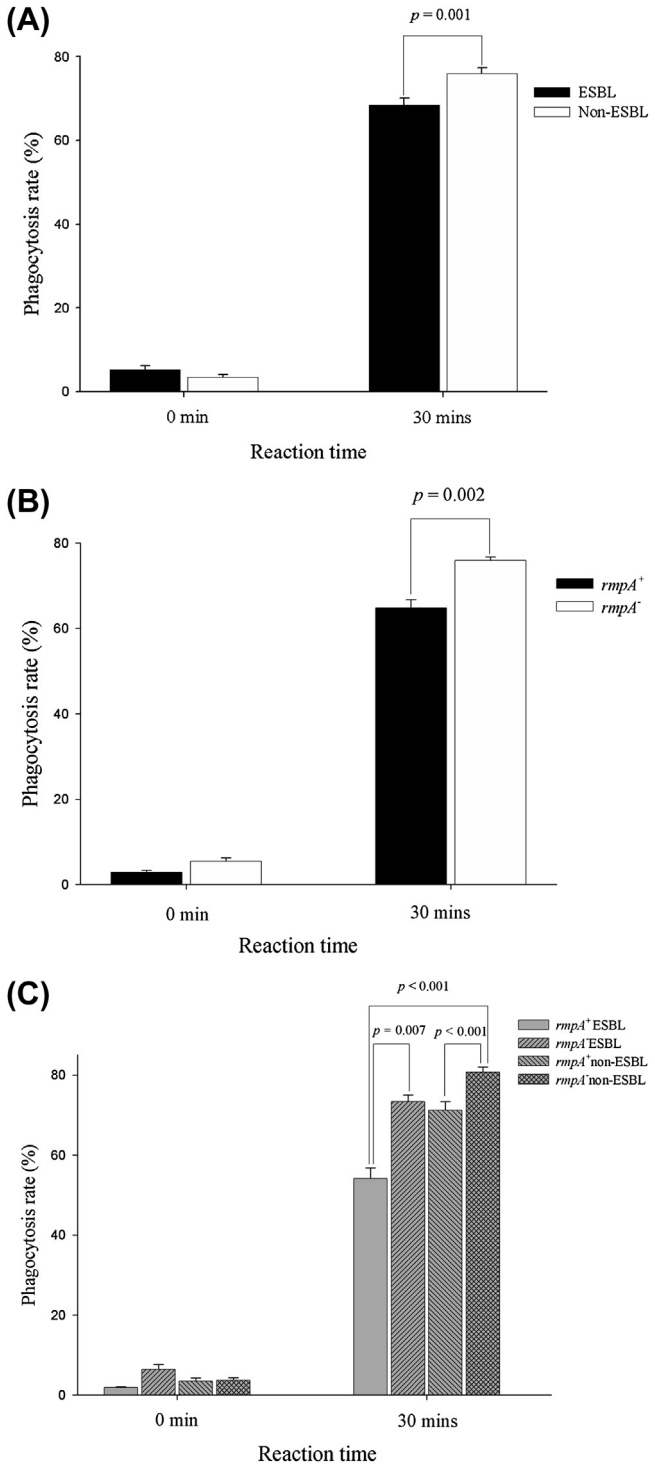

Figure 2. The graph shows (A) the comparison between neutrophil phagocytosis of extended-spectrum beta-lactamase (ESBL)-producing strains ($n = 28$) and non-ESBL-producing strains ($n = 28$; $p = 0.001$); (B) the comparison between neutrophil phagocytosis of $rmpA^+$ strains ($n = 21$) and $rmpA^-$ strains ($n = 35$; $p = 0.002$); and (C) the comparison between neutrophil phagocytosis of $rmpA^{+/-}$ ESBL-producing and $rmpA^{+/-}$ non-ESBL-producing *Klebsiella pneumoniae*. Resistance is more significant in $RmpA^+$ ESBL-producing strains ($n = 7$) than in $rmpA^-$ ESBL-producing strains ($n = 21$; $p = 0.007$), $rmpA^+$ non-ESBL-producing strains ($n = 14$), and $rmpA^-$ non-ESBL-producing strains ($n = 14$; $p < 0.001$). No statistically significant differences exist between $rmpA^-$ ESBL-producing strains and $rmpA^+$ non-ESBL-producing strains ($p = 0.9$).

was conducted with different strains selected from the study groups. The results were similar for several experiments. Each group with 10^6 CFU (Fig. 3A) and 10^7 CFU (Fig. 3B) of one different strains by randomly selection of $rmpA^+$ ESBL-KP, $rmpA^+$ non-ESBL-KP, $rmpA^-$ ESBL-KP, and $rmpA^-$ non-ESBL-KP was inoculated intraperitoneally into BALB/c mice. The mice were observed for survival for 14 days (Fig. 3). Mice infected with 10^6 CFU of $rmpA^+$ strains of non-ESBL-KP/ESBL-KP had a higher mortality rate (94.4%/50%), compared to infection by $rmpA^-$ strains of non-ESBL-KP/ESBL-KP (94.4%/50% vs. 0%/0%; $p < 0.001$). Mice infected with $rmpA^-$ ESBL-KP strains and $rmpA^-$ non-ESBL-KP strains all survived for 14 days (Fig. 3A). Mice infected with 10^7 CFU of $rmpA^+$ ESBL-KP strains had a higher mortality rate (88.9%) than mice with $rmpA^-$ ESBL-KP strains (0%) and $rmpA^-$ non-ESBL-KP strains (16.6%; $p = 0.003$). No statistically significant differences of mortality rate existed between the $rmpA^-$ ESBL-KP strains and the $rmpA^-$ non-ESBL-KP strains (0% vs. 16.6%, respectively; $p = 0.6$; Fig. 3B). In mice inoculated with 10^7 CFU of *K. pneumoniae*, the survival rates between mice infected with $rmpA^+$ ESBL-KP strains and mice infected with $rmpA^+$ non-ESBL-KP strains were the same (88.9% vs. 94.4%, respectively). In addition, mice infected with ESBL $rmpA^+$ strains or non-ESBL $rmpA^+$ strains had a lower survival rate than mice infected with $rmpA^-$ strains, which suggests that *rmpA* contributes to virulence. The study was conducted with different strains selected from the study groups. The results were similar for several experiments.

Discussion

In this study we investigated the interaction and relationship between ESBL-KP and the *rmpA* genes of *K. pneumoniae* on serum resistance, neutrophil phagocytosis, and animals *in vivo*. The ESBL-KP strains proved to be significantly more resistant to serum killing, compared to the non-ESBL-KP strains. Furthermore, our results showed an additive effect of the *rmpA* genes on ESBL-KP in the serum resistance assay. Based on the neutrophil phagocytosis reaction, the resistance was greater in $rmpA^+$ ESBL-KP strains than in $rmpA^+$ non-ESBL-KP strains, $rmpA^-$ ESBL-KP strains, and $rmpA^-$ non-ESBL-KP strains. Invasion of the blood stream by microorganisms is partly a function of the ability to evade the bactericidal effect of serum, which is mediated by the complement cascade.[31] Because serum and neutrophil bacteriocidal activities may be innate immunity factors in host pathogenicity, *rmpA* genes in ESBL-KP with superior resistance to serum and neutrophils hypothetically indicate a greater pathogenic potential in ESBL-KP strains than in non-ESBL-KP strains of non-K1/K2 *K. pneumoniae*. The results *in vivo* animal experiments were similar to the bioassay findings.

Virulent factors such as capsular antigens, serum resistance, and lipopolysaccharides are involved in the

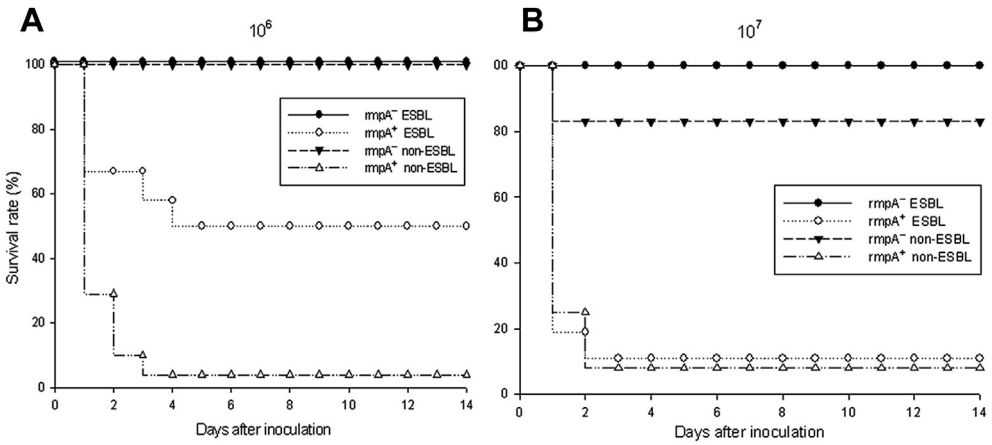

Figure 3. Lethality in BALB/c mice. Each group of Balb/c mice (for each group, $n = 18$) were intraperitoneally inoculated with (A) 10^6 colony-forming units (CFU) and (B) 10^7 CFU of *rmpA*$^{+/-}$ extended-spectrum beta-lactamase (ESBL)-producing and *rmpA*$^{+/-}$ non-ESBL-producing *Klebsiella pneumoniae*. Their survival was followed for 14 days. Mice infected with *rmpA*$^+$ ESBL-producing strains had the most significant mortality rate, compared to *rmpA*$^-$ ESBL-producing strains and *rmpA*$^-$ non-ESBL-producing strains ($p = 0.0003$). No statistically significant differences exist between *rmpA*$^-$ ESBL-producing strains and *rmpA*$^-$ non-ESBL-producing strains ($p = 0.6$; B).

pathogenicity of *K. pneumoniae* infections. Among 77 currently recognized capsular serotypes, K1 and K2 are reportedly associated with virulence and infection. An epidemiologic study demonstrates that the capsular serotypes K1 and K2 are prevalent in invasive *K. pneumoniae* syndrome.[28] For *K. pneumoniae*, some investigations have shown that lipopolysaccharide molecules determine serum sensitivity.[32–34]

According to a previous study, the *rmpA* gene is positively associated with the mucoid phenotype in all strains of *K. pneumoniae* isolated from invasive liver abscesses and the bloodstream, regardless of the capsular serotype.[35] The *rmpA* gene types that harbor virulent effects on capsular serotypes K1/K2 are unknown. However, the present study revealed that most *rmpA*$^+$ ESBL-KP strains with resistance to innate immunity, including serum killing and neutrophil phagocytosis, was confirmed by cellular and animal models. Additional experiments will be needed to clarify whether *rmpA* gene types have an effect on capsular serotype K1/K2 infections.

A major limitation of this study was that we did not use isogenic strains to study the impact of the *rmpA* gene on ESBL-KP virulence. The knock-out of the *rmpA* gene in the ESBL-KP and complementary experiments need further demonstration of evidence of this conclusion.

In summary, the expression of the mucoviscosity phenotype by the presence of the *rmpA* gene is a generally virulent factor in infections involving non-K1/K2 ESBL-KP strain infections. We conclude that the pathogenic potential is greater in *rmpA*$^+$ ESBL-KP strains than in *rmpA*$^-$ ESBL-KP strains and non-ESBL-KP strains.

Conflicts of interest

Conflicts of interest: All authors declare no conflicts of interest.

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