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Clinical Features and Risk Factors Associated With 30-Day Mortality in Patients With Pneumonia Caused by Hypervirulent *Klebsiella pneumoniae* (hvKP)

Jeong-Hwan Hwang, M.D.[1,2], Mallikarjun Handigund, Ph.D.[3], Joo-Hee Hwang, M.D.[1,2], Yong Gon Cho, M.D.[2,3], Dal Sik Kim, M.D.[2,3], and Jaehyeon Lee, M.D.[2,3]

[1]Department of Internal Medicine, Jeonbuk National University Medical School and Hospital, Jeonju, Korea; [2]Research Institute of Clinical Medicine of Jeonbuk National University-Biomedical Research Institute of Jeonbuk National University Hospital, Jeonju, Korea; [3]Department of Laboratory Medicine, Jeonbuk National University Medical School and Hospital, Jeonju, Korea

Background: Reports on metastatic or invasive infections by hypervirulent *Klebsiella pneumoniae* (hvKP) have increased recently. However, the effects of its virulence on clinical course and outcomes in pneumonia patients have rarely been addressed. We assessed and compared the clinical features of hvKp and classic *K. pneumoniae* (cKP) strains isolated from patients with pneumonia caused by *K. pneumoniae*. We also investigated the effects of virulence factors and the *K. pneumoniae* capsular serotypes K1 and K2 on mortality.

Methods: In this retrospective study, we enrolled 91 patients diagnosed as having pneumonia caused by *K. pneumoniae* and obtained their demographic and clinical data from medical records. We evaluated genes for K1 and K2, antimicrobial susceptibility, and the virulence genes *rmpA, iutA, entB, ybtS, kfu, mrkD*, and *allS*. Strains that possessed *rmpA* and *iutA* were defined as hvKP (N=39), while the remaining were classified as cKP (N=52). Odds ratio (OR) for the risk factors associated with 30-day mortality was calculated using the binary logistic regression model.

Results: The 30-day mortality in all patients was 23.1%; it was 17.9% (7/39) in the hvKP group and 26.9% (14/52) in the cKP group (P=0.315). Bacteremia (OR=38.1; 95% confidence interval [CI], 2.5–570.2), altered mental status (OR=8.8; 95% CI, 1.7–45.0), and respiratory rate >30 breaths/min (OR=4.8; 95% CI, 1.2–20.0) were independent risk factors for 30-day mortality in all patients.

Conclusions: Our results suggest that hypervirulence determinants do not have a significant effect on 30-day mortality in patients with pneumonia caused by *K. pneumoniae*.

Key Words: Hypervirulence, *Klebsiella pneumoniae*, Pneumonia, Mortality

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Corresponding author:
Jaehyeon Lee, M.D., Ph.D.
Department of Laboratory Medicine, Jeonbuk National University Medical School and Hospital, 20 Geonji-ro, Deokjin-gu, Jeonju 54907, Korea
Tel: +82-63-250-2693
Fax: +82-63-250-1200
E-mail: laboratorymedicine@gmail.com

INTRODUCTION

Klebsiella pneumoniae, a leading cause of healthcare-associated infection, is an opportunistic pathogen that causes infection in immunocompromised and hospitalized patients [1]. *K. pneumoniae* is the second most common cause of community-acquired pneumonia (CAP) in Asia and the most common gram-negative pathogen [2]. In hospital-acquired pneumonia (HAP), including ventilator-associated pneumonia (VAP), *K. pneumoniae* is the second most common gram-negative pathogen [3].

In the 1980s, a clinical syndrome characterized by endophthalmitis associated with liver abscess due to community-acquired

K. pneumoniae was described in Taiwan [4]. *K. pneumoniae* can be classified as classic *K. pneumoniae* (cKP) or hypervirulent *K. pneumoniae* (hvKP); hvKP differs from cKP in its clinical and phenotypic characteristics [5, 6]. cKP strains have been associated with infections involving the urinary tract, lungs, abdominal cavity, intravascular regions, surgical sites, and soft tissue and have been identified as the cause of subsequent bacteremia in hospitals and long-term care facilities [6]. In contrast, hvKP strains have been associated with invasive and metastatic infections, including pyogenic liver abscesses, bacteremia, and necrotizing fasciitis, in healthy adults and diabetes patients [7].

At least 78 capsular polysaccharide serotypes exist in *K. pneumoniae* [6]. For hvKP strains, eight capsular serotypes have been described to date, namely, K1, K2, K5, K16, K20, K54, K57, and KN1 [6]. According to recent reports, the K1 and K2 serotypes are mainly associated with hvKP, and virulence factors, such as *rmpA*, aerobactin, *kfu*, and *allS*, are more dominant in hvKP than in cKP [8, 9]. However, few studies have explored the clinical characteristics and 30-day mortality in view of the virulence factors or K1 and K2 serotypes, which are closely related to pneumonia caused by hvKP [10, 11]. To our knowledge, this is the first study to examine the effects of the K1 and K2 serotypes and the virulence genes *rmpA, iutA, entB, ybtS, kfu, mrkD*, and *allS* on 30-day mortality in patients with pneumonia caused by *K. pneumoniae*.

MATERIALS AND METHODS

Study setting and data collection

This was a retrospective study conducted at Jeonbuk National University Hospital, Jeonju, Korea. All patients (aged ≥19 years) were diagnosed as having pneumonia caused by *K. pneumoniae* between January 2014 and December 2014. During the study period, a total of 91 patients with pneumonia due to *K. pneumoniae* were identified (men, 65 [71.4%]; mean age, 67.7±14.4 years). All the laboratory tests were performed in routine clinical practice. All samples intentionally collected for this study were cultured in blood agar and MacConkey agar plates in a 35°C incubator for 16–24 hours and identified using the Vitek MS system (BioMérieux, Hazelwood, MI, USA). All antimicrobial susceptibility tests (ASTs) were performed using Vitek 2 AST 211 cards (BioMérieux, Marcy-l'Étoile, France) and interpreted using the VITEK 2 identification system, according to the Clinical and Laboratory Standards Institute M100 S16:2016 guidelines [12]. All procedures were performed according to the manufacturer's instructions. After routine practice, all isolates were stored in skim milk in a deep freezer at -80°C. When there were multiple strains of *K. pneumoniae* in a respiratory sample (sputum, bronchoalveolar lavage, and endotracheal aspirate), only the first isolated strain was analyzed. We retrospectively reviewed the medical records of the patients and collected the following data: age, sex, vital signs, underlying diseases, type of pneumonia, concomitant bacteremia, clinical signs and symptoms, laboratory findings, antimicrobial used for the episode of pneumonia, and AST results. The primary clinical outcome was 30-day mortality. We selected the associated risk factors on 30-day mortality based on a review of the literature on pneumonia and clinicians' experiences prior to medical records review. The protocol for this study was approved by the Institutional Review Board of Jeonbuk National University Hospital (IRB No. 2017-12-022), which waived the requirement of obtaining informed consent from patients.

Definitions

HAP was defined as pneumonia occurring ≥48 hours after hospital admission that did not meet all of the healthcare-associated pneumonia (HCAP) criteria [13]. HCAP was defined as pneumonia in patients with more than one of the following risk factors for infection by multidrug-resistant bacteria: hospitalization for two or more days in the past 90 days; residency in a nursing home or long-term care facility; receipt of antimicrobial treatment, chemotherapy, or wound care within 30 days before the current infection; receipt of hemodialysis; and receipt of home wound care [13]. CAP was defined as pneumonia that developed outside the hospital, long-term care facility, or nursing home setting that did not meet the HCAP criteria [13, 14]. hvKP was defined based on positivity for both *rmpA* and *iutA* [9], and the remaining cases were classified as cKP. Appropriate antimicrobial treatment was defined as the use of antibiotics with activity against the target pathogen within 24 hours after diagnosis of pneumonia [13].

Microbiological evaluation of virulence genes and capsular serotypes

We extracted DNA from stored *K. pneumoniae* strains by the boiling method [15], using a DNA extraction buffer (Seegene, Seoul, Korea). Two or three loopfuls of colonies on blood agar plates were transferred into an Eppendorf tube containing 1 mL of distilled water and 100 µL of DNA extraction solution. After vortexing, the solution was boiled at 95°C for 20 minutes and centrifuged at 15,000×*g* in a microcentrifuge for 10 minutes. The supernatant was used as the template for multiplex PCR to

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detect the K1 and K2 capsular serotypes and virulence genes. Primer sets for *magA* (*wzy*-like polymerase specific to K1 strains), *wzi* (the gene specifying the K2 capsular serotype), and other virulence genes (*rmpA, iutA, entB, ybtS, kfu, mrkD*, and *allS*) were described previously [16].

Statistical analysis

Descriptive statistics included frequency analysis (percentages) for categorical variables and mean±standard deviation or median (range) for continuous variables. We analyzed categorical variables using chi-square test or Fisher's exact test; we analyzed continuous variables using Student's t-test or Mann–Whitney U-test, as appropriate. $P<0.05$ was considered statistically significant, and all probability values were two-tailed. We performed univariate and multivariate logistic regression analyses to evaluate the risk factors for 30-day mortality in patients with pneumonia caused by *K. pneumoniae*. Variables that showed a significant difference ($P<0.1$) in the univariate analysis were included in the multivariate analysis. All statistical analyses were conducted with MedCalc Statistical Software ver.19.2.1. (MedCalc Software Ltd., Ostend, Belgium).

RESULTS

Clinical features of the patients

The baseline characteristics of the patients are presented in Table 1. hvKP strains were obtained from 39 (42.9%) patients and cKP strains from 52 (57.1%) patients. In all patients, chronic lung disease (N=44, 48.4%) was the most common underlying disease, followed by cerebrovascular disease (N=39, 42.9%), and CAP, HCAP, and HAP accounted for 40.7% (N=37), 24.2% (N=22), and 37.4% (N=34), respectively. Patients harboring cKP strains had a significantly higher prevalence of malignancy than those harboring hvKP strains (30.8% vs 12.8%, $P=0.044$).

Table 1. Baseline characteristics of patients with pneumonia caused by *Klebsiella pneumoniae*

Characteristic	hvKP (N=39)	cKP (N=52)	*P*
Age (yr)	68±14	67±15	0.681
Males	27 (69.2)	38 (73.1)	0.688
Underlying diseases			
Diabetes mellitus	12 (30.8)	14 (26.9)	0.688
Malignancy	5 (12.8)	16 (30.8)	**0.044**
Chronic lung disease	17 (43.6)	27 (51.9)	0.431
Chronic kidney disease	12 (30.8)	10 (19.2)	0.203
Chronic heart disease	6 (15.4)	11 (21.2)	0.485
Chronic liver disease	3 (7.7)	4 (7.7)	1.000
Cerebrovascular disease	17 (43.6)	22 (42.3)	0.903
Charlson's comorbidity index	6.0±3.1	6.5±3.6	0.519
Type of pneumonia			
Community-acquired pneumonia	17 (43.6)	20 (34.6)	0.384
Healthcare-associated pneumonia	7 (17.9)	15 (28.8)	0.230
Hospital-acquired pneumonia	15 (38.5)	19 (36.5)	0.851
Clinical features at diagnosis of pneumonia			
Altered mental status	16 (41.0)	23 (44.2)	0.760
Respiratory rate >30 breaths/min	5 (12.8)	11 (21.2)	0.301
Heart rate >125 beats/min	9 (23.1)	12 (23.1)	1.000
Arterial pH <7.35	9 (23.1)	10 (19.2)	0.655
Blood urea nitrogen level >10.71 mmol/L	13 (33.3)	16 (30.8)	0.795
Sodium level <130 mmol/L	5 (12.8)	6 (11.5)	1.000
Hematocrit <0.3 fraction	19 (48.7)	22 (42.3)	0.543
Shock at diagnosis	4 (10.3)	8 (15.4)	0.474
Bacteremia	1 (2.6)	5 (9.6)	0.232
Initial appropriate antibiotics	32 (82.1)	50 (96.2)	**0.035**
30-day mortality	7 (17.9)	14 (26.9)	0.315

Data are presented as mean±SD or number (%).

Microbiological characteristics and antimicrobial susceptibility

Table 2 compares the frequencies of capsular serotypes and virulence factors between hvKP and cKP strains. The capsular serotypes K1 and K2 accounted for 15.4% (14/91) and 17.6% (16/91) of all *K. pneumoniae* strains, respectively. hvKP strains had a significantly higher prevalence of the K1 serotype (35.9% vs 0%, $P<0.001$), *allS* (38.5% vs 3.8%, $P<0.001$), *kfu* (35.9% vs 11.5%, $P=0.005$), and *ybtS* (76.9% vs 46.2%, $P=0.003$) than cKP strains. hvKP strains had a significantly higher median number of virulence factors than cKP strains ($P<0.001$) .

All 91 *K. pneumoniae* strains presented uniform susceptibility to ampicillin. There was no significant difference in the antimicrobial susceptibility rate, except for susceptibility to ampicillin-sulbactam (Table 2). Although the percentage of extended-spectrum β-lactamase (ESBL)-producing cKP strains was higher than that of ESBL-producing hvKP strains, the difference was not statistically significant (25.6% vs 34.6%, $P=0.359$).

Risk factors associated with 30-day mortality in patients with pneumonia caused by *K. pneumoniae*

The overall 30-day mortality in patients with pneumonia caused by *K. pneumoniae* was 23.1% (21/91). As shown in Table 3,

Table 2. Frequencies of capsular serotypes, virulence factors, and antimicrobial susceptibility between hvKP and cKP strains

Variable	hvKP (N=39)	cKP (N=52)	*P*
Capsular serotype			
K1	14 (35.9)	0 (0)	**<0.001**
K2	10 (25.6)	6 (11.5)	0.080
Non K1/K2	15 (38.5)	46 (88.5)	**<0.001**
Virulence factor			
entB	39 (100)	49 (94.2)	0.257
ybtS	30 (76.9)	24 (46.2)	**0.003**
kfu	14 (35.9)	6 (11.5)	**0.005**
mrkD	38 (97.4)	49 (94.2)	0.632
allS	15 (38.5)	2 (3.8)	**<0.001**
Number of virulence factors	3 (2–5)	3 (0–5)	**<0.001**
Antimicrobial susceptability			
Amikacin	34 (87.2)	43 (82.7)	0.557
Ampicillin-sulbactam	28 (71.8)	25 (48.1)	0.023
Aztreonam	28 (71.8)	29 (55.8)	0.118
Cefazolin	27 (69.2)	27 (51.9)	0.096
Cefepime	28 (71.8)	29 (55.8)	0.118
Cefotaxime	28 (71.8)	28 (53.8)	0.082
Cefoxitin	30 (76.9)	39 (75.0)	0.832
Ceftazidime	28 (71.8)	27 (51.9)	0.055
Ertapenem	35 (89.7)	44 (84.6)	0.474
Gentamicin	34 (87.2)	40 (76.9)	0.214
Levofloxacin	29 (74.4)	29 (55.8)	0.068
Meropenem	39 (100)	48 (92.3)	0.132
Piperacillin-tazobactam	29 (74.4)	30 (57.7)	0.099
Tigecycline	37 (94.9)	45 (86.5)	0.291
ESBL	10 (25.6)	18 (34.6)	0.359

Data are presented as median (range) or number (%).
Abbreviations: hvKP, hypervirulent *Klebsiella pneumoniae*; cKP, classic *K. pneumoniae*; ESBL, extended-spectrum β-lactamase; NA, not available.

bacteremia (P=0.002), shock at diagnosis (P=0.005), altered mental status (P<0.001), high respiratory rate (P=0.002), heart rates >125 beats/min (P<0.001), arterial pH<7.35 (P=0.001), blood urea nitrogen >10.71 mmol/L (P=0.001), and hematocrit <0.3 fraction (P=0.023) were significantly associated with 30-day mortality. Multivariate analysis showed that bacteremia, altered mental status, and respiratory rates >30 breaths/min were independent risk factors for 30-day mortality in all patients (Tables 3 and 4).

Table 3. Clinical variables associated with 30-day mortality in patients with pneumonia caused by *Klebsiella pneumoniae*

Characteristic	Death (N=21)	Survival (N=70)	*P*
Age (yr)	68±14	68±15	0.961
Male sex	17 (81.0)	48 (68.6)	0.271
Underlying diseases			
Diabetes mellitus	5 (23.8)	21 (30.0)	0.582
Malignancy	7 (33.3)	14 (20.0)	0.241
Chronic lung disease	10 (47.6)	34 (48.6)	0.939
Chronic kidney disease	6 (28.6)	16 (22.9)	0.592
Chronic heart disease	3 (14.3)	14 (20.0)	0.753
Chronic liver disease	2 (9.5)	5 (7.1)	0.660
Cerebrovascular disease	9 (42.9)	30 (42.9)	1.000
Charlson's comorbidity index	7.0±3.3	6.0±3.4	0.253
Type of pneumonia			
Community-acquired pneumonia	5 (23.8)	30 (42.9)	0.116
Healthcare-associated pneumonia	6 (28.6)	16 (22.9)	0.592
Hospital-acquired pneumonia	10 (47.6)	24 (34.3)	0.268
Serotype			
K1	2 (9.5)	12 (17.1)	0.508
K2	3 (14.3)	13 (18.6)	0.756
Non K1/K2	16 (76.2)	45 (64.3)	0.309
Virulence factor			
entB	20 (95.2)	68 (97.1)	0.549
ybtS	13 (61.9)	41 (58.6)	0.785
kfu	5 (23.8)	15 (21.4)	0.773
mrkD	20 (95.2)	67 (95.7)	1.000
allS	3 (14.3)	14 (20.0)	0.753
Number of virulence factors	3 (2-5)	3 (0-5)	0.871
Hypervirulent *K. pneumoniae*	7 (33.3)	32 (45.7)	0.315
Clinical features at diagnosis of pneumonia			
Altered mental status	17 (81.0)	22 (31.4)	**<0.001**
Respiratory rate >30 breaths/min	9 (42.9)	7 (10.0)	**0.002**
Heart rate >125 beats/min	12 (57.1)	9 (12.9)	**<0.001**
Arterial pH <7.35	10 (47.6)	9 (12.9)	**0.001**
Blood urea nitrogen level >10.71 mmol/L	13 (61.9)	16 (22.9)	**0.001**
Sodium level <130 mmol/L	5 (23.8)	6 (8.6)	0.118
Hematocrit <0.3 fraction	14 (66.7)	27 (38.6)	**0.023**
Shock at diagnosis	7 (33.3)	5 (7.1)	**0.005**
Bacteremia	5 (23.8)	1 (1.4)	**0.002**
Initial appropriate antibiotics	18 (85.7)	64 (91.4)	0.426

Data are presented as mean±SD or median (range) or number (%).

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Table 4. Multivariate analysis of 30-day mortality in the patients with pneumonia caused by *Klebsiella pneumoniae*

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Bacteremia	21.6 (2.4 –197.5)	0.007	38.1 (2.5–570.2)	0.008
Altered mental status	9.3 (2.8–30.8)	<0.001	8.8 (1.7–45.0)	0.009
Respiratory rate >30 breaths/min	6.8 (2.1–21.6)	0.001	4.8 (1.2–20.0)	0.031
Blood urea nitrogen level >10.71 mmol/L	5.5 (1.9–15.6)	0.001		

Abbreviations: OR, odds ratio; CI, confidence interval.

DISCUSSION

This study aimed to assess and compare the clinical features of hvKP and cKP strains isolated from patients with pneumonia caused by *K. pneumoniae* and to investigate the effect of virulence factors and the K1 and K2 serotypes on mortality. This study is significant because, unlike other studies [10, 11], it compared hvKP and cKP considering all types of pneumonia caused by *K. pneumoniae*, including CAP, HCAP, and HAP. Our results demonstrated that the presence of virulence factors and the K1 and K2 serotypes did not affect mortality, whereas bacteremia, altered mental status, and respiratory rates >30 breaths/min were independent risk factors for mortality.

There were no significant risk factors associated with hvKP infection compared with cKP infection. Malignancy was associated with a significantly higher risk for cKP infection, which is consistent with the fact that hospitalized cancer patients are highly susceptible to cKP infection [7]. cKP is the primary strain of nosocomial infection, unlike hvKP, which is associated with CAP [7]. However, we found no significant difference between hvKP and cKP in CAP, HCAP, and HAP patients. In line with this finding, a study conducted in China revealed no difference between hvKP and cKP in HAP caused by *K. pneumoniae* [17].

Studies in Asia, Europe, and America showed that K1 and K2 have a close relationship with hvKP [5]. In our study, K1 and K2 serotypes were present in 61.5% of hvKP; this frequency was significantly higher than that of cKP. In two recent studies on VAP caused by *K. pneumoniae*, the prevalence rates of K1 and K2 serotypes in hvKP were 70.6% and 64.3%, respectively. Further, in bacteremic CAP, the prevalence rate of the K1 and K2 serotypes in hvKP was 53.1%, which was higher than that in cKP (46.9%) [10, 14, 18]. The prevalence of K1 and K2 is high in hvKP because strains of these serotypes are more resistant to phagocytosis and intracellular elimination by macrophages and neutrophils than are strains of other serotypes [19, 20]. In addition, a study on VAP reported that the number of virulence factors associated with hvKP is significantly higher than that associated with cKP [18].

In this study, the 30-day mortality in all patients with pneumonia due to *K. pneumoniae* was 23.1% (21/91). It was 14.3% (5/35) for CAP, 27.3% (6/22) for HCAP, 29.4% (10/34) for HAP, and 19.3% (11/57) for community-onset pneumonia (CAP and HCAP). The mortality associated with community-onset pneumonia caused by *K. pneumoniae* in this study was lower than that in Taiwan (CAP, 25.5%; HCAP, 34.1%; and community-onset pneumonia, 29.3%) [11]. However, in this study, the mortality due to *K. pneumoniae*-associated CAP was higher than the previous data (7.3%) due to total pathogens [2]. The mortality due to HAP was similar to the finding (25–58%) reported in a previous study [3].

There was no significant difference in mortality associated with the hvKP and cKP strains in our study. Moreover, the K1 and K2 serotypes and the virulence genes (*rmpA, iutA, entB, ybtS, kfu, mrkD*, and *allS*) were not independent risk factors for mortality. In univariate analysis, the unadjusted odds ratio (OR) of hvKP mortality was 0.6 (95% confidence interval [CI], 0.2–1.7). After adjustment for confounding variables in multivariate logistic regression analysis, the adjusted OR of hvKP was 0.8 (95% CI, 0.2–2.9) (data not shown). Therefore, hvKP was not associated with mortality. Similar results have been reported previously [10, 11, 14]. There was no significant difference in mortality between hvKP- and cKP-associated bloodstream infections [21, 22]. However, the *pks* gene cluster has been found to affect mortality in bloodstream infections caused by *K. pneumoniae*, and further studies on other virulence factors that affect mortality and on the relationships between virulence factors that contribute to mortality are warranted [23].

ASTs revealed no significant difference between the hvKP and cKP strains, except for susceptibility to ampicillin-sulbactam. In previous studies, resistance to third-generation cephalosporins in hvKP-associated VAP were 0–10% [18], 14.3% [24], and 30–40% [10]. In this study, the resistance rate of hvKP to cefo-

taxime was 29.2%. However, antibiotic resistance in hvKP may need monitoring as the prevalence of antibiotic-resistant-hvKP is increasing gradually, and early antibiotic administration is important for survival of pneumonia patients [25-27].

Our study had several limitations. First, it was a retrospective, single center study conducted in a small population. However, it is larger than the populations in previous studies on pneumonia due to *K. pneumoniae* [16, 17, 24]. Second, we could not evaluate all the risk factors that would be accessible in an observational study. Third, a standardized definition of hvKP has not yet been established. Although definitions of hvKP differ across studies, *iuc* and either *rmpA* or *rmpA2* should be considered a key virulence factor of hvKP. In this study, of the 41 strains positive for *iutA*, 39 harbored *rmpA*. However, we did not analyze the presence of *rmpA2* in two strains. Finally, we investigated only some virulence factors and tested none of the virulence factors in an animal study. We cannot exclude the possibility of inconsistencies between virulence experiments conducted by different groups.

In conclusion, to our knowledge, this is the first study comparing the influence of hvKp and cKP on the mortality in patients with pneumonia caused by *K. pneumoniae*. Our study is of value in investigating the relationship between bacterial virulence and the clinical outcome of pneumonia. We report that hvKP strains do not have a clinically significant effect on mortality in these patients. Future studies are required to confirm the interactions between related virulence factors and the role of other virulence factors in the clinical outcomes of pneumonia caused by *K. pneumoniae*.

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AUTHOR CONTRIBUTIONS

Jeong-H H and JHL designed the study, analyzed the data, and wrote the manuscript. Jeong-H H and Joo-H H contributed to data collection and ensuring the integrity of the data. JHL designed the microbiological tests, and MH and JHL conducted the microbiological tests. Jeong-H H contributed to statistical data analysis and interpretation. Jeong-H H and JHL were the major contributors to the study conception and manuscript revision. YGC and DSK contributed to the manuscript revision. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that no conflict of interest exists.

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ORCID

Jeong-Hwan Hwang https://orcid.org/0000-0002-2897-2202
Mallikarjun Handigund https://orcid.org/0000-0002-6496-4437
Joo-Hee Hwang https://orcid.org/0000-0002-8616-3411
Yong Gon Cho https://orcid.org/0000-0003-3011-6875
Dal Sik Kim https://orcid.org/0000-0001-7467-2086
Jaehyeon Lee https://orcid.org/0000-0003-3211-8903

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