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Namikawa, H., Niki, M., Niki, M. et al. Clinical and virulence factors related to the 30-day mortality of Klebsiella pneumoniae bacteremia at a tertiary hospital: a case–control study. *European Journal of Clinical Microbiology & Infectious Diseases*. 38, 2291–2297. (2019).
doi:10.1007/s10096-019-03676-y

Original article

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Acknowledgements: This research was supported by the Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency Development, AMED [Grant number JP 17fk0108208, 18fk0108052h0002 and 19fk0108094] and JSPS KAKENHI [Grant number 16K09939 and 19K16650].

Abstract

Purpose: Klebsiella pneumoniae bacteremia is a critical clinical presentation that is associated with high mortality. However, extremely few studies have investigated the virulence factors related to mortality of *K. pneumoniae* bacteremia in patients. The present study elucidated clinical and virulence factors associated with the 30-day mortality of *K. pneumoniae* bacteremia at a tertiary hospital.

Methods: The medical records of 129 patients with *K. pneumoniae* bacteremia admitted to Osaka City University Hospital between January 2012 and December 2018 were retrospectively reviewed. Patient background characteristics, antimicrobial regimens, and prognosis were evaluated. Additionally, virulence factors were assessed using multiplex polymerase chain reaction to elucidate their association with *K. pneumoniae*.

Results: The 30-day mortality was 10.9% in patients with *K. pneumoniae* bacteremia. The male-to-female ratio, age, and underlying disease did not differ between the non-survivor and survivor groups. Multivariate analysis showed that sepsis [odds ratio (OR), 7.46; $p = 0.005$] and *iutA* (OR, 4.47; $p = 0.046$) were independent predictors associated with the 30-day mortality of *K. pneumoniae* bacteremia.

Conclusion: Despite the relatively low 30-day mortality of patients with *K. pneumoniae* bacteremia, the treatment of those with sepsis and those infected with *K. pneumoniae* harboring *iutA* may require careful management for improving their outcomes.

Keywords: bacteremia; *iutA*; *Klebsiella pneumoniae*; mortality; sepsis

Introduction

Klebsiella pneumoniae is a common gram-negative pathogen in community- or nosocomial-acquired infections. It causes urinary tract and surgical site infections, pneumonia, and bloodstream infections [1]. Particularly, *K. pneumoniae* bacteremia is a clinically critical presentation associated with high mortality. Previous studies have indicated that mortality was approximately 16%–40% in patients with *K. pneumoniae* bacteremia [2-4]. Therefore, elucidating the predictors associated with mortality in *K. pneumoniae* bacteremia is critical in clinical practice.

A new hypervirulent variant of *K. pneumoniae* has recently emerged, mainly in the Asian Pacific Rim [5]. In recent years, hypervirulent *K. pneumoniae* (hvKP) infections, which have spread globally, are increasingly recognized as a critical clinical issue worldwide. HvKP can lead to life-threating community-acquired infections including bacteremia, liver abscesses, endophthalmitis, and meningitis [5]. We have previously reported two serious cases of hvKP infection that caused multiple organ abscesses and endophthalmitis [6].

Moreover, hvKP strains are characterized by their distinct sticky phenotype on agar plates. Therefore, this hypermucoviscous phenotype has widely been recognized to be strongly related to its hypervirulence, and the term “hypermucoviscous” has often been used as a synonym of “hypervirulence.” However, a recent study suggested that the hypermucoviscosity and hypervirulence were two distinct phenotypes that should not be used as synonyms [7]. The hypervirulence of *K. pneumoniae* should be defined by its genetic

background and should not solely rely on the bacterial phenotype. In other words, hypermucoviscosity determined by the string test is inadequate to suggest the hypervirulence of the isolated strain. Several studies have reported that the hvKP strain is strongly associated with mortality in *in vivo* mouse infection models [8,9]. However, studies focusing on the virulence factors related to mortality caused by *K. pneumoniae* bacteremia in humans are scarce [2].

The present study aimed to investigate clinical variables and virulence factors associated with the 30-day mortality of *K. pneumoniae* bacteremia at a tertiary hospital.

Materials and Methods

The medical records of 129 patients with *K. pneumoniae* bacteremia who had been admitted to Osaka City University Hospital between January 2012 and December 2018 were retrospectively reviewed. Patient characteristics including age, sex, underlying disease, clinical features, therapies, and prognosis were evaluated. In cases where *K. pneumoniae* had been isolated on multiple occasions within the 6-year period in the same patient, only the first episode of *K. pneumoniae* bacteremia was reviewed. The present study was approved by the Ethics Committee of Osaka City University, and the thesis was approved on Mar 22, 2019, with approval number 4299. The need for written informed consent was waived owing to the retrospective nature of the study.

Definition and source of bacteremia

Bacteremia was defined as the presence of one or more positive blood cultures in patients with clinical signs of infection such as fever, shaking chills, and sweats, with or without local signs and symptoms. The diagnosis of biliary tract *K. pneumoniae* infection was based on the presence of three or more of the following clinical and diagnostic findings: 1) fever and/or chills; 2) laboratory evidence of an inflammatory response; 3) jaundice or abnormal liver chemistry; 4) biliary dilation or the evidence of an etiology observed on imaging; and 5) *K. pneumoniae* isolated from a bile specimen. Patients were diagnosed with urinary tract infection (UTI) due to *K. pneumoniae* in the presence of two or more of the following clinical and diagnostic findings: 1) *K. pneumoniae* confirmed in a urine specimen, 2) clinical manifestations suggestive of UTI, and 3) imaging findings suggestive of pyelonephritis. The following characteristic symptoms and urinary findings were considered to define UTI: dysuria, suprapubic pain, hematuria, flank pain, costovertebral angle tenderness, nausea or vomiting, and pyuria or bacteriuria [10]. Furthermore, the following imaging findings were considered to support a UTI diagnosis: perinephric stranding, renal swelling, Gerota's fascia thickening, and poor segmental enhancement region [11]. *K. pneumoniae* pneumonia was diagnosed in the presence of new persistent pulmonary infiltrates, which were not otherwise explained, appearing on chest radiographs along with purulent respiratory secretions and systemic signs of an inflammatory response [12]. Catheter-related bloodstream infection due to *K. pneumoniae* was diagnosed based on one or more of the following clinical and

diagnostic findings: 1) *K. pneumoniae* growth was detected in at least one percutaneous blood and catheter-tip culture and 2) *K. pneumoniae* growth was detected in a blood sample obtained from a catheter hub at least 2 h before its growth was detected in a peripheral vein blood sample [13].

Assessment of laboratory data

In patients with a positive initial blood culture, the leukocyte count as well as C-reactive protein and albumin levels were assessed within 2 days of the culture. The 2016 Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) criteria were used in the present study.

Identification of bacteria

All *K. pneumoniae* isolates were identified via colony morphologic analysis and Gram staining. Isolate identification, antimicrobial susceptibility, and minimum inhibitory concentrations (MICs) were confirmed using a MicroScan WalkAway-96 SI instrument (Beckman Coulter, Brea, CA, USA). The results were interpreted using the 2018 Clinical and Laboratory Standards Institute (CLSI) criteria. The production of extended-spectrum beta-lactamase (ESBL) was screened by measuring the MICs of cefotaxime, ceftazidime, and aztreonam. Confirmatory testing was performed using an Ambler class C & ESBL Identification Set (Kanto Chemical, Tokyo, Japan). The production of carbapenemase was

screened using the sodium mercaptoacetate inhibition test. All plates were incubated at 35°C for 24 h.

Antimicrobial therapies

The attending physician determined the appropriate initial antimicrobial therapy. Antimicrobial therapy administered within 5 days after bacteremia onset was defined as empirical therapy, whereas that administered thereafter as definitive therapy. Appropriate empirical or definitive antibiotic therapy was defined as that matching the *in vitro* susceptibility results according to the CLSI criteria [14].

Virulence factors

The following nine virulence genes were assessed using multiplex polymerase chain reaction (PCR), as previously described [15]: *magA* (specific to K1 capsule serotype) and *rmpA* for mucoviscosity; *entB*, *ybtS*, *kfu*, and *iutA* for siderophore pathway; *mrkD* for adhesins; *allS* for allantoin metabolism; and *wzi* for the specific K2 capsular serotype. Primers used for multiplex PCR are listed in Table 1.

Statistical analysis

Patient characteristics, blood test data, therapies, and bacterial characteristics were compared between the survivor and non-survivor groups. The Mann–Whitney *U* and chi-square tests

were used for the univariate comparison of categorical data. To determine the independent predictors of the 30-day mortality of *K. pneumoniae* bacteremia, variables with a *p* value <0.1 in the univariate analyses were considered for inclusion in the multivariate logistic regressions using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 3.5.3). EZR is a modified version of R commander (version 2.4-0) that includes the statistical functions frequently used in biostatistics. A *p* value of <0.05 was considered statistically significant.

Results

Clinical characteristics and laboratory findings

The clinical characteristics and laboratory findings of the non-survivors and survivors of *K. pneumoniae* bacteremia are summarized in Table 2. The 30-day mortality in patients with *K. pneumoniae* bacteremia was 10.9%. The non-survivor group (n = 14) included 8 males and 6 females, with a mean age of 66.2 years. The survivor group (n = 115) included 72 males and 43 females, with a mean age of 66.5 years. The non-survivor group included 8 (61.5%), 3 (23.1%), and 5 (35.7%) patients with malignancy, diabetes mellitus, and nosocomial-acquired bacteremia, respectively. Conversely, the survivor group included 72 (62.6%), 40 (34.8%), and 62 (53.9%) patients with malignancy, diabetes mellitus, and nosocomial-acquired bacteremia, respectively. The Charlson score did not differ between the non-survivor and

survivor groups (4.1 ± 2.9 vs. 3.4 ± 2.6, $p = 0.37$). However, sepsis was significantly more frequent among patients in the non-survivor group than those in the survivor group (78.6% vs. 26.1%, $p < 0.001$).

Therapies

Table 3 summarizes the empirical and definitive therapies for patients with *K. pneumoniae* bacteremia in the non-survivor and survivor groups. The most frequently used antibiotics for both empirical and definitive therapy were carbapenems in both the non-survivor and survivor groups (empirical: 64.3% and 33.0%, respectively; definitive: 28.6% and 24.8%, respectively). The rates of appropriate empirical or definitive therapy did not differ between both the non-survivor and survivor groups (empirical: 100% and 94.8%, respectively, $p = 0.84$; definitive: 100% and 96.5%, respectively, $p = 1.00$).

Bacterial characteristics

Bacterial characteristics observed in the non-survivor and survivor groups are shown in Table 4. Although 10 (8.7%) *K. pneumoniae* isolates in the survivor group were ESBL-producing strains, none of the isolates in the non-survivor group were ESBL-producing strains. Notably, the frequency of the *iutA* was higher in the non-survivor group than the survivor group (35.7% vs. 9.6%, $p = 0.02$), whereas the frequency of other virulence genes was not significantly different between the two groups.

Prognostic factors

Multivariate analysis (Table 5) revealed that sepsis [odds ratio (OR), 7.46; $p = 0.005$] and *iutA* (OR, 4.47; $p = 0.046$) were independent predictors of the 30-day mortality of *K. pneumoniae* bacteremia.

Discussion

Our single-center, case-control study including 129 patients with *K. pneumoniae* bacteremia revealed that the 30-day mortality in patients with *K. pneumoniae* bacteremia was 10.9%, which was lower than that previously reported [2-4]. Second, it was suggested that sepsis and *iutA* are independent predictors of the 30-day mortality of *K. pneumoniae* bacteremia.

The relatively lower 30-day mortality of 10.9% compared with previous studies might be attributable to several factors. First, most previous studies have reported that nosocomial *K. pneumoniae* infections were associated with a higher mortality compared with community-acquired *K. pneumoniae* infections [16-18]. Furthermore, nosocomial infections due to *K. pneumoniae* bacteremia were more frequent than community-acquired infections due to *K. pneumoniae* bacteremia, ranging from 55% to 92% [4,16-18]. On the other hand, the rate of nosocomial *K. pneumoniae* infections in the present study was 52%, which was lower than that previously reported; this lower rate might be associated with the lower mortality observed in the present study. Second, a previous study has reported that

administering the appropriate empirical antibiotic therapy was an important predictor of patient outcomes [19]. Furthermore, previous reports have demonstrated that the rate of appropriate antibiotic therapy administered for *K. pneumoniae* bacteremia varied from 46% to 85% [2,4,17,19]. In contrast, the rate of appropriate empirical therapy in the present study was 95%, which was prominently higher than the previously reported rates. The frequency of resistant bacteria might therefore contribute to the differences in mortality. Therefore, these findings support that the 30-day mortality in the present study was lower than that reported by previous studies.

K. pneumoniae is one of the most frequently isolated bacteria from patients with sepsis and is known to harbor various virulence factors [20]. The capsule of *K. pneumoniae* has multiple different functions: it prevents phagocytosis and the functioning of antimicrobial peptides, blocks complement-mediated lysis and opsonization, and averts the inflammatory response. Considering the diverse roles of the capsule, *K. pneumoniae* infections are associated with a potentially severe clinical presentation. Moreover, *K. pneumoniae* endotoxin, which is produced by other gram-negative bacteria as well, induces cytokines and leads to organ dysfunction and sepsis [21]. These findings suggest that sepsis is associated with an increased risk of mortality in patients with *K. pneumoniae* bacteremia.

Bacteria have developed the iron acquisition tools termed as siderophores to compete with the host [22]. In addition to its iron acquisition function, siderophores can modulate host immune responses [23], enhance bacterial dissemination [24], and regulate the virulence

factor production [25]. Aerobactin, a citrate-hydroxamate siderophore, is produced by several pathogenic gram-negative bacteria to help iron assimilation. Aerobactin has increasingly been recognized for its key role in mediating the enhanced virulence of hvKP. Recently, Russo *et al*. showed that aerobactin is a key factor that distinguished hvKP from non-hvKP strains [26]. Aerobactin gene, along with *rmpA*, was frequently isolated from *K. pneumoniae* bacteremic strains that caused primary liver abscess [27]. Moreover, aerobactin is identified as an important virulence factor associated with mortality in an *in vivo* mouse infection model [9]. *iutA* encodes for the outer-membrane receptor for ferric aerobactin. Vargas et al. reported that *iutA* promotes biofilm formation [28]. Moreover, Tang et al. showed that *iutA* is an independent pathogenicity factor for abscess formation [29]. A few clinical studies have reported that *iutA* is associated with a high pathogenicity of *K. pneumoniae* [30,31]. Together, these findings suggest that *iutA* is associated with an increased risk of mortality in patients with *K. pneumoniae* bacteremia. However, we did not examine the other siderophore-related genes, such as *iucA* or *iroB*. In the future, these genes should be analyzed to determine their association with the mortality of *K. pneumoniae* bacteremia.

The present study has several limitations. First, the study population was relatively small, and the study was conducted in one tertiary hospital only; therefore, there was selection bias. Future studies including more patients with *K. pneumoniae* bacteremia in both community and tertiary hospital settings are required to address this limitation. Second, we examined only nine virulence genes in *K. pneumoniae*, and additional virulence genes of *K.*

pneumoniae that have been reported to date should be analyzed to determine their association with the mortality of *K. pneumoniae* bacteremia. Third, the primary aim of this retrospective case-control study was to elucidate the predictive factors of mortality caused by *K. pneumoniae* bacteremia; however, prospective studies are required to further clarify these factors.

In conclusion, the present study demonstrated that the 30-day mortality in patients with *K. pneumoniae* bacteremia was relatively low at 10.9%. Sepsis and *iutA* may be independent predictors of the 30-day mortality of *K. pneumoniae* bacteremia. Therefore, the treatment of patients with bacteremia caused by *K. pneumoniae* in the state of sepsis and of those infected with *K. pneumoniae* harboring *iutA* may require careful management for improving their outcomes.

Conflicts of interest: The authors declare that there are no conflicts of interest.

Funding: This research was supported by the Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency Development, AMED [Grant number JP 17fk0108208, 18fk0108052h0002 and 19fk0108094] and JSPS KAKENHI [Grant number 16K09939 and 19K16650].

Ethical approval: The study was approved by the Ethics Committee of Osaka City University, and the thesis was approved on Mar 22, 2019, with approval number 4299.

Informed consent: Not applicable to this study.

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Table 1. Primers used for multiplex PCR

Primer	Target gene	Sequence (*5′→3′*)	Reference
magA-F	*magA*	GGTGCTCTTTACATCATTGC	32
magA-R	*magA*	GCAATGGCCATTTGCGTTAG	32
rmpA-F	*rmpA*	CATAAGAGTATTGGTTGACAG	1
rmpA-R	*rmpA*	CTTGCATGAGCCATCTTTCA	1
entB-F	*entB*	GTCAACTGGGCCTTTGAGCCGTC	1
entB-R	*entB*	TATGGGCGTAAACGCCGGTGAT	1
ybtS-F	*ybtS*	GACGGAAACAGCACGGTAAA	1
ybtS-R	*ybtS*	GAGCATAATAAGGCGAAAGA	1
kfu-F	*kfu*	GGCCTTTGTCCAGAGCTACG	1
kfu-R	*kfu*	GGGTCTGGCGCAGAGTATGC	1
iutA-F	*iutA*	GGGAAAGGCTTCTCTGCCAT	1
iutA-R	*iutA*	TTATTCGCCACCACGCTCTT	1

mrkD-F	*mrkD*	AAGCTATCGCTGTACTTCCGGCA	1
mrkD-R	*mrkD*	GGCGTTGGCGCTCAGATAGG	1
allS-F	*allS*	CATTACGCACCTTTGTCAGC	1
allS-R	*allS*	GAATGTGTCGGCGATCAGCTT	1
wzi-F	*wzi*	CAACCATGGTGGTCGATTAG	33
wzi-R	*wzi*	TGGTAGCCATATCCCTTTGG	33

Table 2. Clinical characteristics and laboratory findings of non-survivor and survivor patients with *K. pneumoniae* bacteremia

Variables	Non-survivors (n = 14)	Survivors (n = 115)	*p* value
Age (years) [a]	66.2 ± 11.1	66.5 ± 12.9	0.84 [b]
Male	8 (57.1%)	72 (62.6%)	0.92 [c]
Underlying disease [d]			
Malignancy	8 (61.5%)	72 (62.6%)	1.00 [c]
Immunosuppressive drug or corticosteroid use	1 (7.7%)	19 (16.5%)	0.67 [c]
Diabetes mellitus	3 (23.1%)	40 (34.8%)	0.59 [c]
Chronic obstructive pulmonary disease	3 (23.1%)	12 (10.4%)	0.37 [c]
Chronic kidney disease	2 (15.4%)	24 (20.9%)	0.92 [c]
Lower gastrointestinal disease	0 (0%)	7 (6.1%)	0.79 [c]
Charlson score [a,d]	4.1 ± 2.9	3.4 ± 2.6	0.37 [b]
Use of antibiotics prior to isolation [d,e]	6 (46.2%)	60 (52.2%)	0.91 [c]
Leukocyte count ≥ 12000 (/μL)	8 (57.1%)	41 (35.7%)	0.20 [c]
C-reactive protein ≥ 10 (mg/dL)	10 (71.4%)	53 (46.1%)	0.09 [c]
Albumin ≤ 2.5 (g/dL)	7 (50.0%)	27 (23.5%)	0.07 [c]
Nosocomial infection	5 (35.7%)	62 (53.9%)	0.32 [c]
Operation within 30 days [d]	1 (7.7%)	11 (9.6%)	1.00 [c]
Hospitalization within 90 days [d]	4 (30.8%)	48 (41.7%)	0.64 [c]
Infection site			
Biliary tract	5 (35.7%)	37 (32.2%)	1.00 [c]
Urinary tract	1 (7.1%)	20 (17.4%)	0.55 [c]
Lung	3 (21.4%)	9 (7.8%)	0.24 [c]
Intravascular device	0 (0%)	6 (5.2%)	0.84 [c]
Others	0 (0%)	4 (3.5%)	1.00 [c]
Unknown	5 (29.2%)	38 (33.0%)	1.00 [c]

Abscess [f]	2 (16.7%)	11 (9.6%)	0.79 [c]
Multiple	1 (7.1%)	1 (0.87%)	0.52 [c]
Sepsis	11 (78.6%)	30 (26.1%)	<0.001 [c]

[a] Data are presented as means ± standard deviation

[b] Mann–Whitney U test

[c] Chi-square test

[d] Details were not known for one patient in the non-survivor group

[e] 60 days prior to isolation

[f] Details were not known for two patients in the non-survivor group

Table 3. Empirical and definitive therapies in the non-survivor and survivor groups of patients with *K. pneumoniae* bacteremia

Variables	Empirical therapy		Definitive therapy	
	Non-survivors (n = 14)	Survivors (n = 115)	Non-survivors (n = 7)[a]	Survivors (n = 113)[b]
Carbapenems	9(64.3%)	38 (33.0%)	2 (28.6%)	28 (24.8%)
Tazobactam/Piperacillin	3 (21.4%)	20 (17.4%)	0 (0%)	19 (16.8%)
Quinolones	0 (0%)	7 (6.1%)	1 (14.3%)	11 (9.7%)
Fourth-generation cephalosporins	1 (7.1%)	11 (9.6%)	0 (0%)	9 (8.0%)
Third-generation cephalosporins	1 (7.1%)	20 (17.4%)	1 (14.3%)	14 (12.4%)
Sulbactam/ampicillin	0 (0%)	8 (7.0%)	2 (28.6%)	13 (11.5%)
Second-generation cephalosporins	0 (0%)	9 (7.8%)	0 (0%)	7 (6.2%)
First-generation cephalosporins	0 (0%)	1 (1.7%)	1 (14.3%)	12 (10.6%)
Appropriate therapy	14 (100%)	109 (94.8%)	7 (100.0%)	109 (96.5%)

[a] Seven patients died before definitive therapy.

[b] One patient was transferred to a different hospital, and one patient discontinued the treatment before definitive therapy.

Table 4. Bacterial characteristics of the non-survivor and survivor groups of patients with *K. pneumoniae* bacteremia

Variables	Non-survivors (n = 14)	Survivors (n = 115)	*p* value [a]
Beta-lactamase production			
ESBL	0 (0%)	10 (8.7%)	0.60
AmpC	0 (0%)	0 (0%)	NA
carbapenemase	0 (0%)	1 (0.9%)	1.00
Virulence gene			
mucoviscosity			
magA (specific to K1 capsule serotype)	5 (35.7%)	56 (48.7%)	0.53
rmpA	4 (28.6%)	13 (11.3%)	0.17
siderophore			
entB	14 (100.0%)	112 (97.4%)	1.00
ybtS	3 (21.4%)	35 (30.4%)	0.70
kfu	4 (28.6%)	35 (30.4%)	1.00
iutA	5 (35.7%)	11 (9.6%)	0.02
adhesin			
mrkD	14 (100.0%)	112 (97.4%)	1.00
allantoin metabolism			
allS	1 (7.1%)	5 (4.3%)	1.00
other			
wzi (specific to K2 capsule serotype)	3 (21.4%)	10 (8.7%)	0.31

[a] Chi-square test

ESBL, extended-spectrum beta-lactamase; NA, not available

Table 5. Multivariate analysis of predictors associated with the 30-day mortality of *K. pneumoniae* bacteremia

Predictor	OR (95% CI)	*p* value
C-reactive protein ≥ 10 (mg/dL)	1.54 (0.39–6.03)	0.53
Albumin ≤ 2.5 (g/dL)	2.60 (0.70–9.64)	0.15
Sepsis	7.46 (1.85–30.1)	0.005
iutA	4.47 (1.03–19.5)	0.046

CI, confidence interval; OR, odds ratio