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ORIGINAL ARTICLE

# Pathogenic significance of hemorrhagic pneumonia in hematologic malignancy patients with *Stenotrophomonas maltophilia* bacteremia: clinical and microbiological analysis

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## Abstract

Hemorrhagic pneumonia (HP) is known as the clinical manifestation of *Stenotrophomonas maltophilia* infection, while catheter-related blood stream infection (CRBSI) is the common clinical presentation of *S. maltophilia* bacteremia (SMB). The purpose of this study is to evaluate the risk factors for mortality in hematologic malignancy patients with SMB and to analyze clinical and microbiological characteristics of HP associated with SMB and CRBSI. SMB cases of patients with a hematologic malignancy were collected from 2006 through 2016. The overall 30-day mortality rate and mortality risk factors were assessed. The expression of major virulence-associated genes from *S. maltophilia* isolates, which included genes encoding type-1 fimbriae (*smf-1*), proteases (*StmPr1* and *StmPr2*), and esterase (*Smlt3773*), from the blood of patients with HP and CRBSI was investigated. The phenotypic and genotypic traits were also compared. A total of 118 cases of SMB were included. The overall 30-day mortality rate was 61.0%. A multivariable analysis showed that HP was the most important risk factor for mortality (adjusted OR = 106.41; 95% CI = 5.18–2184.55). Although no statistical significance was observed in microbiological analysis, isolates from HP have a trend toward a higher protease activity (93.8% vs. 73.3%, $P = 0.172$). Clinical analysis showed that thrombocytopenia ($P = 0.037$) and prolonged neutropenia ($P = 0.043$) were significant factors associated with HP. Our data, which includes hematologic malignancy patients with SMB, suggest that HP is the significant risk factor for mortality and that the unique characteristics of patients and microbes contribute to the pathogenesis.

**Keywords** *Stenotrophomonas maltophilia* · Bacteremia · Hemorrhagic pneumonia · Hematologic malignancy · Protease

## Introduction

*Stenotrophomonas maltophilia* has emerged as a significant pathogen with high mortality, especially in debilitated patients [1]. Since the 1990s, several studies have reported increasing incidence of *Stenotrophomonas maltophilia* infection [2, 3]. An appropriate antibiotic treatment can improve the clinical outcome; however, the intrinsic resistance to several antibiotics makes it difficult to determine the appropriate antibiotics [4–6]. Trimethoprim/sulfamethoxazole (TMP/SMX) has been shown to be the most potent antibiotic for *S. maltophilia* infection, but its resistance to TMP/SMX has been increasing in recent decades [7].

The most common clinical manifestations of *S. maltophilia* are pneumonia and blood stream infection [1]. Pneumonia with bacteremia is associated with high mortality compared to catheter-related blood stream infection (CRBSI) [8, 9]. Furthermore, case studies of patients who died with hemorrhage pneumonia (HP), which is a very characteristic clinical presentation of *S. maltophilia* infection, have been reported [10–20]. Several virulence factors have been proposed that could be related to pathogenesis and clinical manifestation of *S. maltophilia*. Most notably, *S. maltophilia* produces a

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protease encoded by *StmPR1* [21]. It is presumed that this protease is involved in the damage of lung tissue and eventually may cause pulmonary hemorrhage [22, 23]. The ability to form a biofilm is also expected to contribute to pathogenicity [24].

In this study, we identified cases of *S. maltophilia* bacteremia in patients with hematologic malignancy and analyzed clinical outcomes and mortality risk factors. Representative clinical manifestations were also taken into consideration as a mortality risk factor. To evaluate which microbiological and/or clinical factors contributed to clinical manifestation, patients with HP and CRBSI and blood isolates of *S. maltophilia* were collected. Clinical and microbiological characteristics were compared.

## Methods

### Study population

The study was conducted at the Samsung Medical Center, Seoul, South Korea, from January 2006 through December 2016. The institution is a 1950-bed tertiary care university-affiliated hospital and referral center combined with the 700-bed Samsung Comprehensive Cancer Center. Patients ≥ 18 years of age with hematologic malignancy who were diagnosed with *S. maltophilia* bacteremia were identified through their electronic medical records. These cases and data were included as part of our prior study [25]. Only cases where *S. maltophilia* was cultured from more than two separate blood samples or was cultured from a single blood sample in patients with concomitant clinical symptoms and focus could be included as true bacteremia [26]. Each patient was included only once during the study period. The 30-day mortality rate was calculated. All cases were divided into 30-day survivor or non-survivor groups to evaluate risk factors for mortality.

### Variables and clinical information definitions

We collected the following data from electronic medical records: age, gender, length of hospital stay, polymicrobial infection, previously isolated pathogens, breakthrough infection during carbapenem therapy, underlying diseases, history of hematopoietic stem cell transplantation, Charlson comorbidity index (CCI) [27], neutropenia, administration of chemotherapy, use of mechanical ventilation, use of central venous catheter, history of renal replacement therapy, sequential organ failure assessment (SOFA) score [28], previous antibiotics use, and appropriate empirical antibiotics use.

Clinical presentation of *S. maltophilia* bacteremia was classified as catheter-related infection (CRI), pneumonia, soft-tissue infection, intra-abdominal infection, or other. CRI included not only CRBSI but also central line–associated blood stream infection (CLABSI). CRBSI was defined as culture of the same organism from a catheter hub and peripheral blood sample meeting criteria of differential time to positivity. CLABSI was defined as a primary blood stream infection (BSI) associated with the presence of a central venous catheter the time of or within 48 h before onset of the BSI without any evidence of other explainable infection focus [29, 30]. Pneumonia was defined when cultures of the blood, sputum, trans-tracheal aspirate, or the bronchoalveolar lavage fluid were positive along with concomitant clinical respiratory symptoms [17]. Pneumonia patients who had hemoptysis and/or significant bleeding from the endotracheal or tracheal tube were defined as HP; other pneumonia patients lacking those symptoms were defined as non-hemorrhagic pneumonia (NHP).

If two or more bacterial and/or fungal pathogens were isolated from a patient's blood sample, it was defined as a polymicrobial infection. Any antibiotic administered via an intravenous route for more than 48 h within a 30-day period was noted as previous antibiotic use. Appropriate empirical antibiotic use was defined if the antibiotics were effective against *S. maltophilia* via an in vitro susceptibility test that had been administered within 72 h after obtaining the blood culture sample [31].

### Blood culture and drug sensitivity test

All blood samples were taken from a peripheral vein and/or a central line. A Bactec-9240 system (Becton Dickinson, Sparks, MD) or a BacT/Alert 3D system (bioMérieux Inc., Marcy l'Etoile, France) was used for blood cultures. A MicroScan Neg Combo Panel Type 63 (Siemens Inc., USA) was used to identify microbes and their antimicrobial agent sensitivity in conjunction with a standard identification card and the modified broth microdilution method.

### Microbiological analysis

The presence of virulence-associated genes that encode type-1 fimbriae (*smf-1*), major and minor extracellular proteases (*StmPr1* and *StmPr2*), and esterase (encoded by the *Smlt3773* locus in the genome of strain K279a) was determined by PCR. Genes were amplified with 125 specific primer pairs that were designed to the genomic sequence of the *S. maltophilia* strain 126 K279a [24]. Each gene was amplified separately, and amplicons were identified based on their expected fragment size [22]. Sequences were subjected to BLAST analysis and compared to the genome of *S. maltophilia* reference strain K279a. Protease activity was also evaluated as described previously [32]. In protease assay, the presence of clear halos around bacterial growth was taken as evidence of protease activity.

**Table 1** Clinical characteristics of *Stenotrophomonas maltophilia* bacteremia in patients with a hematologic malignancy and the factors associated with 30-day mortality

| | Overall (118) | 30-day mortality | | | | |
|---|---|---|---|---|---|---|
| | | Univariate analysis | | | Multivariable analysis | |
| | | Survivor (46) | Non-survivor (72) | *P* value | Odd ratio (95% CI) | *P* value |
| Age (mean ± SD) | 55.68 ± 13.12 | 52.30 ± 13.30 | 57.83 ± 12.63 | 0.025[b] | | |
| Sex (male) | 69 (58.5) | 26 (56.5) | 43 (59.7) | 0.731 | | |
| Hospital stay ≥ 30 days | 52 (44.1) | 12 (26.1) | 40 (55.6) | 0.002[b] | | |
| Polymicrobial infection | 40 (33.9) | 10 (21.7) | 30 (41.7) | 0.026[b] | | |
| Previous *S. maltophilia* isolation | 43 (36.4) | 10 (21.7) | 33 (45.8) | 0.008[b] | | |
| Focus of infection | | | | | | |
| CRI | 51 (43.2) | 31 (67.8) | 20 (27.8) | < 0.001[b] | | |
| Pneumonia | 55 (46.6) | 8 (21.4) | 47 (65.3) | < 0.001 | | |
| Hemorrhagic | 20 (16.9) | 1 (2.2) | 19 (26.4) | 0.001[b] | 106.41 (5.18–2184.55) | 0.002 |
| Non-hemorrhagic | 35 (29.7) | 7 (15.2) | 28 (38.9) | 0.006[b] | 9.83 (2.24–43.20) | 0.002 |
| SSTI | 4 (3.4) | 2 (4.3) | 2 (2.8) | 0.642 | | |
| IAI | 6 (5.1) | 4 (8.7) | 2 (2.8) | 0.207 | | |
| Underlying malignancy | | | | | | |
| Leukemia | 81 (68.6) | 36 (78.3) | 45 (62.5) | 0.072[b] | | |
| Lymphoma | 25 (21.2) | 6 (13.0) | 19 (26.4) | 0.084[b] | | |
| Allogenic HPSCT | 21 (17.8) | 6 (13.0) | 15 (20.8) | 0.281 | | |
| Refractory or recurrent | 62 (52.5) | 18 (39.1) | 44 (61.1) | 0.020[b] | | |
| Underlying comorbidities | | | | | | |
| CCI (median, IQR) | 2 (2–3) | 2 (2–2) | 2 (2–3) | 0.331 | | |
| CCI ≥ 3 | 36 (30.5) | 8 (17.4) | 28 (38.9) | 0.013[b] | | |
| Neutropenia | 105 (89.0) | 39 (82.6) | 67 (93.1) | 0.077[b] | | |
| Central venous catheter | 112 (94.9) | 44 (95.7) | 68 (94.4) | 1.000 | | |
| ICU stay | 29 (24.6) | 2 (4.3) | 27 (37.5) | < 0.001[b] | | |
| Mechanical ventilation | 29 (24.6) | 1 (2.2) | 28 (38.9) | < 0.001[b] | | |
| RRT | 17 (14.4) | 1 (2.2) | 16 (22.2) | 0.002[b] | | |
| Initial SOFA score (median, IQR) | 8 (5–14) | 5 (3–6) | 12 (8–16) | < 0.001[b] | 1.91 (1.42–2.58)[a] | < 0.001 |
| Platelet ≤ 20 K/mm$^3$ | 96 (81.3) | 12 (26.1) | 10 (13.9) | 0.097[b] | | |
| Previous antibiotics ≥ 3 | 91 (77.1) | 30 (65.2) | 61 (84.7) | 0.014[b] | | |
| Treatment | | | | | | |
| Appropriate empirical antibiotics use within 72 h | 31 (26.3) | 15 (32.6) | 16 (22.2) | 0.211 | | |
| Early empirical TMP/SMX use within 72 h | 15 (12.7) | 10 (21.7) | 5 (7.0) | 0.020[b] | 0.07 (0.01–0.48) | 0.007 |

Result of Hosmer-Lemeshow goodness of fit test showed non-significance ($P = 0.234$). Result of Nagelkerke square was 0.780. Data are number (%) of patients

*SD*, standard deviation; *CRI*, catheter-related infection; *SSTI*, skin and soft tissue infection; *IAI*, intra-abdominal infection; *HPSCT*, hematopoietic stem cell transplantation; *CCI*, Charlson comorbidity index; *IQR*, interquartile range; *RRT*, renal replacement therapy; *SOFA*, sequential organ failure assessment; *TMP/SMX*, trimethoprim/sulfamethoxazole

[a] The odds ratio indicates that for every 1-point increase in SOFA score

[b] Variable included in multivariable analysis

For the biofilm formation assay, blood *S. maltophilia* isolates and *S. maltophilia* ATCC13637 were used. Microplate assays were performed as previously described with some modifications [33]. Briefly, overnight cultures of *S. maltophilia* were standardized to a 0.5 McFarland standard (equivalent to $1.5 \times 10^8$ CFU/mL) and then diluted (1:100) with fresh LB broth. Aliquots of standardized inocula were added to the wells of sterile flat-bottom 96-well polystyrene tissue culture plates (TPP, Switzerland) and incubated at 37 °C for 24 h in a closed and humidified plastic container. The medium was then discarded, and non-adherent cells were removed by

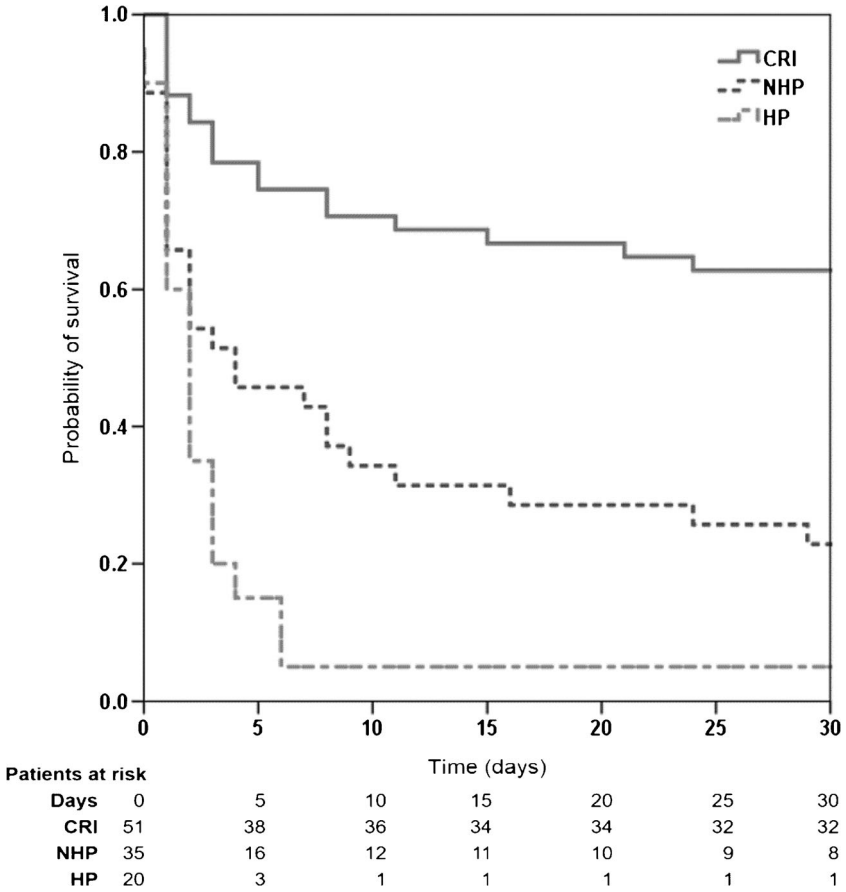


**Fig. 1** Kaplan-Meier curve showing the probability of survival in catheter-related infection (CRI), non-hemorrhagic pneumonia (NPH), and hemorrhagic pneumonia (HP) ($P < .001$)

washing three times in sterilized ultrapure water. The cells were stained with 0.01% crystal violet for 20 min. The excess stain was then removed by washing with water, and the stained biofilms were dried for 30 min at ambient room temperature and extracted with 33% glacial acetic acid. The amount of biofilm produced was quantified by measuring the optical density at 492 nm using a plate reader (Bio-Rad, CA, USA). Non-inoculated medium was used as a control.

Assuming a low cutoff ($OD_c$) represented by three times the SD above the mean OD of the control wells, strains were classified into the following categories: a no biofilm producer ($OD \leq OD_c$), a weak biofilm producer ($OD_c < OD \leq 2 \times OD_c$), a moderate biofilm producer ($2 \times OD_c < OD \leq 4 \times OD_c$), and a strong biofilm producer ($4 \times OD_c < OD$). Each isolate was assayed three times, and the results were presented as the average of the three assays.

The presence of biofilm-associated genes was also investigated. PCR with specific primers for *rmlA*, *spgM*, and *rpfF* was performed as described previously [14]. Amplification conditions were as follows: 30 cycles at 60 °C for 20 s, 72 °C for 30 s, and 94 °C for 20 s.

To evaluate the genomic and epidemiological relatedness of clinical *S. maltophilia* isolates, multilocus sequence typing (MLST) was performed. PCR amplification and sequencing of seven housekeeping genes (*atpD*, *gapA*, *guaA*, *mutM*, *nuoD*, *ppsA*, and *recA*) were included in the MLST scheme and performed as previously described [34]. PCR products were purified with a PCR purification kit (GeneAll Biotechnology, Seoul, Korea) and sequenced at Cosmo Inc. (Seoul, Korea) in both directions under standard conditions. All of the MLST data, including data on new sequence types (STs), have been submitted to the MLST database (http://pubmlst.org/smaltophilia).

## Review of HP cases

For further characterization of HP in *S. maltophilia* infection, we gathered previously reported HP cases [10–20] and HP cases from our study. Descriptive analysis was performed.

## Statistical analysis

All statistical analyses were performed using SPSS 24.0 for Windows (IBM Corp., 2017). A Student *t* test or Mann-Whitney test was used to compare continuous variables, and the chi-squared test or Fisher's exact test was used to compare categorical variables for identification of mortality factors. Variables of $P < 0.10$ in the univariate analysis along with variables considered potential clinically meaningful were

**Table 2** comparison of patients with *Stenotrophomonas maltophilia* bacteremia and hemorrhagic pneumonia or catheter-related infection

| | Hemorrhagic pneumonia (16)[a] | Catheter-related blood stream infection (15)[a] | *P* value |
|---|---|---|---|
| Patient characteristics | | | |
| Age (age ± SD) | 58.94 ± 10.85 | 47.47 ± 13.78 | 0.011 |
| Sex (male) | 6 (37.5) | 7 (46.7) | 0.605 |
| Length of hospital stay ≥ 30 days | 7 (43.8) | 7 (46.7) | 0.870 |
| Polymicrobial infection | 6 (37.5) | 7 (46.7) | 0.605 |
| Previous *S. maltophilia* isolation | 3 (18.8) | 5 (33.3) | 0.433 |
| Underlying malignancy/comorbidities | | | |
| Leukemia | 13 (81.2) | 8 (53.3) | 0.135 |
| Lymphoma | 2 (12.5) | 4 (26.7) | 0.394 |
| Allogenic HPSCT | 3 (18.8) | 4 (26.7) | 0.685 |
| Malignancy recurrence | 9 (56.2) | 8 (53.3) | 0.870 |
| CCI (IQR) | 2 (2–2.75) | 2 (2–3) | 0.770 |
| Medical condition | | | |
| Neutropenia | 16 (100.0) | 11 (73.3) | 0.043 |
| ICU stay | 2 (12.5) | 4 (26.7) | 0.318 |
| Chemotherapy | 16 (100.0) | 13 (86.7) | 0.226 |
| Central venous catheter | 15 (93.8) | 15 (100) | > 0.999 |
| Mechanical ventilation | 3 (18.8) | 3 (20.0) | > 0.999 |
| Renal replacement therapy | 1 (6.2) | 3 (20.0) | 0.333 |
| SOFA score (IQR) | 10 (6–15.75) | 5 (3–13) | 0.071 |
| Platelet (K/mm$^3$) | 12.5 (7–12.5) | 16 (7–86) | 0.299 |
| Platelet ≤ 20,000/mm$^3$ | 15 (93.8) | 9 (60.0) | 0.037 |
| Previous antibiotics use | 16 (100.0) | 13 (86.7) | 0.226 |
| Piperacillin/tazobactam | 7 (43.8) | 7 (46.7) | 0.870 |
| Cefepime | 10 (62.5) | 6 (40.0) | 0.210 |
| Fluoroquinolone | 5 (31.2) | 2 (13.3) | 0.394 |
| Carbapenem[b] | 16 (100.0) | 10 (66.7) | 0.018 |
| Glycopeptide | 14 (87.5) | 10 (66.7) | 0.220 |
| All-cause 14-day mortality | 15 (93.8) | 3 (20.0) | < 0.001 |

Data are number (%) of patients

[a] Among the 20 HP and 19 CRBSI cases, 16 isolates from HP and 15 from CRBSI were collected for further clinical and microbiological analyses

[b] All patients who received carbapenem were previously administrated with three or more antibiotics

*SD*, standard deviation; *HPSCT*, hematopoietic stem cell

included in forward stepwise logistic regression model. Time to death of cases with representative clinical manifestations during the 30-day follow-up period was calculated using the Kaplan-Meier method. All *P* values were two-tailed, and *P* values < 0.05 were considered statistically significant.

## Results

### Study population

During the study period, a total of 118 cases of *S. maltophilia* bacteremia in patients with hematologic malignancy were identified. All cases were hospital-acquired or healthcare-associated infection. The most common clinical presentations were pneumonia (55/118, 46.6%) and CRI (51/118, 43.2%). Of the 55 pneumonia cases, 20 were classified as HP, and of the 51 CRI cases, 19 were classified as CRBSI.

### Risk factors for mortality

Clinical factors associated with 30-day mortality were analyzed and are summarized in Table 1. In a univariate analysis, old age, hospital stay ≥ 30 days before onset of bacteremia, polymicrobial infection, previous *S. maltophilia* isolation, pneumonia, refractory or recurrent malignancy, CCI ≥ 3, previous ICU stay, mechanical ventilation, renal replacement therapy, and high SOFA score were associated with increased 30-day overall mortality. CRI and

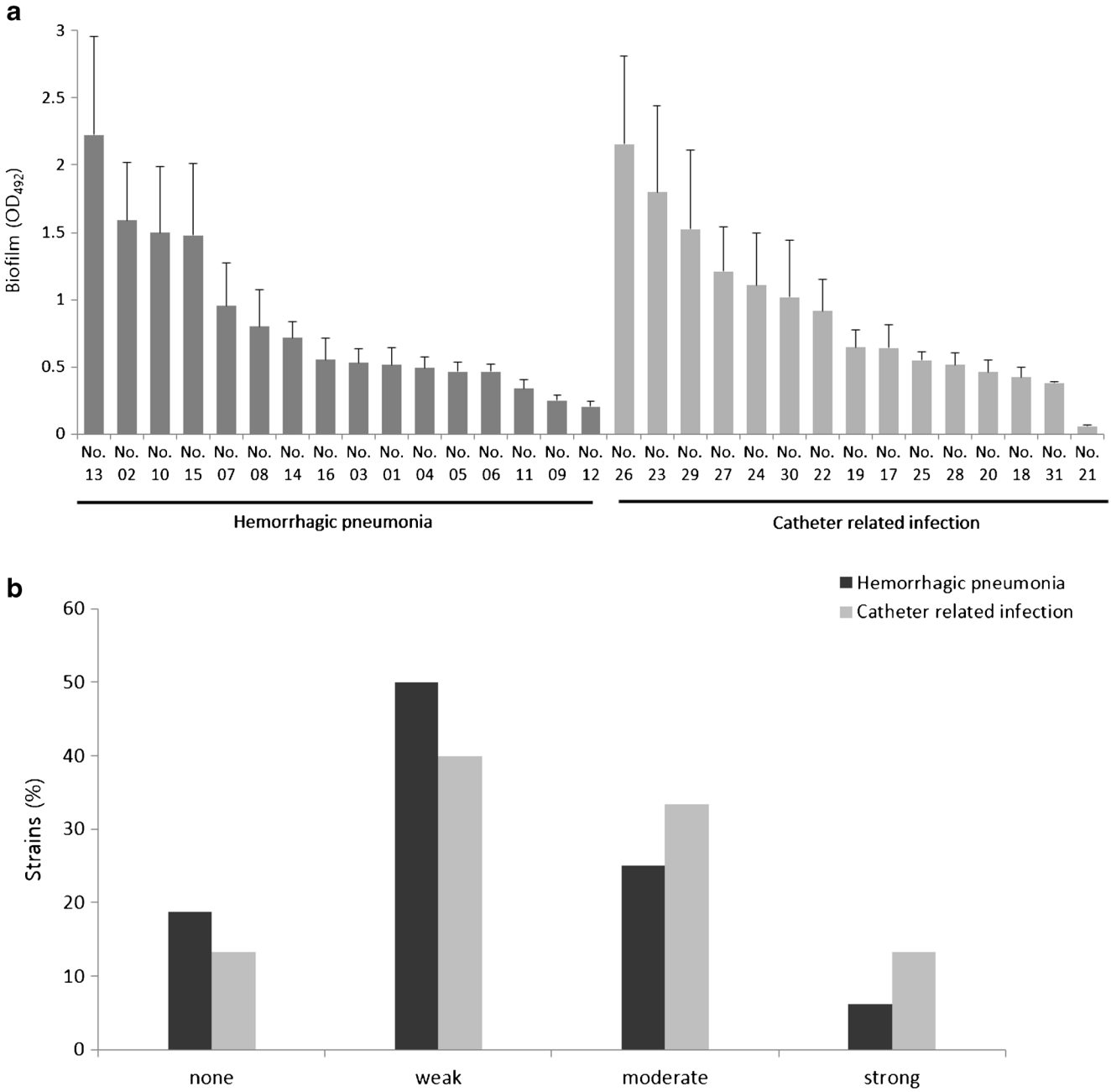


**Fig. 2** Analysis of biofilm formation. **a** Biofilm formation among 31 *Stenotrophomonas maltophilia* isolates from catheter-related bloodstream infections (CRBSIs) and in patients with hemorrhagic pneumonia. The numbering of isolates coincides with the numbering in Table 3. **b** Percentage distribution of CRBSI and hemorrhagic pneumonia isolates belonging to none ($OD_{492} \leq 0.410$; $n = 5$), weak ($0.410 < OD_{492} \leq 0.820$; $n = 14$), moderate ($0.820 < OD_{492} \leq 1.640$; $n = 9$), or strong ($1.640 < OD_{492}$; $n = 3$) biofilm producer groups

early empirical TMP/SMX use within 72 h were found to be associated with favorable outcomes.

In the multivariable analysis, HP was the most important factor of mortality in *S. maltophilia* bacteremia (adjusted odd ratios (aOR) = 106.41, 95% confidence interval (CI) = 5.18–2184.55, *P* value = 0.002), along with high SOFA score (aOR = 1.81, 95% CI = 1.42–2.58, *P* value < 0.001) and NHP (aOR = 9.83, 95% CI = 2.24–43.20, *P* value = 0.002). Early empirical TMP/SMX use within 72 h was associated factors of decreased mortality (aOR = 0.07, 95% CI = 0.01–0.48, *P* value = 0.007).

A Kaplan-Meier curve of mortality for three representative clinical manifestations (CRI, NHP, and HP) of *S. maltophilia* bacteremia in adult patients with hematologic malignancy showed significant survival difference during the 30-day follow-up period ($P < 0.001$, Fig. 1).

**Table 3** microbiological characteristics of *S. maltophilia* bacteremia with hemorrhagic pneumonia and CRBSI

| Isolates no. | Antimicrobial resistance profile | PCR analysis of virulence-associated genes | | | | | Protease activity | ST |
|---|---|---|---|---|---|---|---|---|
| | | *smf-1* | *StmPr1* (1621 bp) | *StmPr1* (868 bp) | *StmPr2* | *Smlt3773* | | |
| HP-01 | | + | + | – | + | + | + | 77 |
| HP-02 | | + | + | – | + | + | + | 77 |
| HP-03 | | + | + | – | + | + | + | 77 |
| HP-04 | CAZ | + | + | + | + | + | + | 24 |
| HP-05 | CAZ, TIC/CV | + | + | – | + | + | + | 102 |
| HP-06 | | + | + | – | + | + | + | 77 |
| HP-07 | | + | + | – | + | + | + | 77 |
| HP-08 | | + | + | + | + | + | + | 229 |
| HP-09 | LEV, CAZ | + | + | + | + | + | + | 24 |
| HP-10 | LEV, CAZ | + | – | + | + | + | + | 210 |
| HP-11 | CAZ | + | + | – | + | + | – | 6 |
| HP-12 | CAZ | + | + | + | + | + | + | 7 |
| HP-13 | LEV, TIC/CV | + | + | + | + | + | + | 219 |
| HP-14 | CAZ, TIC/CV | + | + | – | + | + | + | 91 |
| HP-15 | TIC/CV | + | + | + | + | + | + | 249 |
| HP-16 | SMX/TMP, LEV, CAZ, TIC/CV | + | + | + | + | + | + | 238 |
| CRBSI-17 | | + | + | + | + | + | – | 240 |
| CRBSI-18 | CAZ | + | + | + | + | + | + | 24 |
| CRBSI-19 | CAZ | + | + | – | + | + | + | 31 |
| CRBSI-20 | TIC/CV | + | + | + | + | + | + | 152 |
| CRBSI-21 | CAZ | – | – | – | – | – | – | 77 |
| CRBSI-22 | CAZ | + | + | – | + | + | + | 208 |
| CRBSI-23 | | + | + | + | + | + | + | 239 |
| CRBSI-24 | | + | + | + | + | + | + | 77 |
| CRBSI-25 | | + | + | – | + | + | + | 229 |
| CRBSI-26 | LEV | + | + | – | + | + | – | 28 |
| CRBSI-27 | | + | + | + | + | + | + | 233 |
| CRBSI-28 | TIC/CV | + | + | + | + | + | + | 239 |
| CRBSI-29 | TIC/CV | + | + | – | + | + | + | 229 |
| CRBSI-30 | TIC/CV | + | + | + | + | + | + | 249 |
| CRBSI-31 | LEV, CAZ, TIC/CV | + | + | – | + | + | – | 91 |

*HP*, hemorrhagic pneumonia; *CRBSI*, catheter-related blood stream infection; *CAZ*, ceftazidime; *TIC/CV*, ticarcillin/clavulanic acid; *LEV*, levofloxacin; *SMX/TMP*, sulfamethoxazole/trimethoprim; +, amplification of the expected DNA fragment; −, no amplification; +, presence; −, absence of protease activity; *ST*, sequence type

The 30-day mortality rate was highest in HP, as shown in the multivariable analysis.

## Microbiological analysis

Among the 20 HP and 19 CRBSI cases, 16 isolates from HP and 15 from CRBSI were collected for further clinical and microbiological analyses. Cases with HP showed significantly higher 30-day all-cause mortality compared to those with CRBSI (93.8% vs. 20.0%, $P < 0.001$). Elderly patients, patients with neutropenia ≤ 500/mm3, thrombocytopenia ≤ 20,000/mm3, and carbapenem use were more common in cases with HP (Table 2).

Most of the *S. maltophilia* isolates (26/31, 83.8%) were able to form biofilms similarly in the HP and CRBSI groups (81.3% vs. 86.6%). Percentage of category with no, weak, moderate, or strong biofilm producers was similar in the both groups (Fig. 2). Meanwhile, the prevalence of the *spgM*, *rmlA*, and *rpfF* genes was 83.8%, 51.6%, and 29.0%, respectively. The presence of biofilm-associated genes did not significantly affect the mean amount of biofilm in our study. In general, no significant differences were observed in virulence factors

**Table 4** Comparison of *Stenotrophomonas maltophilia* isolates from patients with hemorrhagic pneumonia or catheter-related infection

| | Hemorrhagic pneumonia (16) | Catheter-related infection (15) | *P* value |
|---|---|---|---|
| Biofilm formation (mean ± SD) | 0.818 ± 0.579 | 0.894 ± 0.581 | 0.607 |
| Category | | | 0.871 |
| Strong | 1 (6.3) | 2 (13.3) | |
| Moderate | 4 (25.0) | 5 (33.3) | |
| Weak | 8 (50.0) | 6 (40.0) | |
| Non-producer | 3 (18.8) | 2 (13.3) | |
| Biofilm-associated genotype | | | |
| *rmlA* | 10 (62.5) | 5 (33.3) | 0.104 |
| *spgM* | 13 (81.2) | 12 (80.0) | > 0.999 |
| *rpfF* | 3 (18.8) | 5 (33.3) | 0.433 |
| Virulence factor | | | |
| *Ssmf-1* | 16 (100.0) | 14 (93.3) | 0.484 |
| *StmPr1 162* bp | 15 (93.8) | 14 (93.3) | > 0.999 |
| *StmPr1 868* bp | 8 (50.0) | 8 (53.3) | 0.853 |
| *StmPr2 1764* bp | 6 (37.5) | 7 (46.7) | 0.605 |
| *Smlt3773 1342* bp | 3 (18.8) | 5 (33.3) | 0.433 |
| Protease activity | 15 (93.8) | 11 (73.3) | 0.172 |

Data are number (%) of patients

between the groups. *S. maltophilia* isolates from HP have a trend toward a higher protease activity (93.8% vs. 73.3%, $P = 0.172$); however, statistical significance was not reached (Tables 3 and 4).

MLST showed high genetic diversity among the *S. maltophilia* clinical isolates. Except for ST77, most STs were represented by one to three *S. maltophilia* isolates. ST77 showed a higher prevalence of isolates with HP than the CRBSI group, although the difference was not significant (31.3% vs. 13.3%, $P = 0.394$).

### Clinical characteristics of hemorrhagic pneumonia in *S. maltophilia* infection

Clinical characteristics of 54 cases of HP-related *S. maltophilia* infections are described in Table 5. Twenty cases of HP were from our data, and 34 cases of HP were from review of papers [10–20]. Mean age of HP patients was 49.1 years old, and the ratio of male to female was approximately 3:2. 96.3% of patients had hematologic malignancy, and acute leukemia was the most frequently underlying hematologic malignancy. Approximately 90% of patients had neutropenia and bacteremia. TMP/SMX prophylaxis was implemented in only 16.7% of the patients. TMP/SMX single or TMP/SMX-fluoroquinolone combination therapy was the most preferred therapy. The three surviving patients were administered TMP/SMX-based therapy. 96.1% of non-surviving patients died within a week. Median time to death was 2 days.

## Discussion

Clinical factors related to mortality in patients with *S. maltophilia* infections have been evaluated in several observational studies. Inadequate empirical antibiotics, admission to the ICU, and septic shock were reported as independent factors associated with mortality [1]. Pneumonia had worse outcomes than CRI in patients with *S. maltophilia* bacteremia [8, 9]. Overall mortality of *S. maltophilia* pneumonia ranged from 23 to 77% and was especially high in patients with a malignancy [1, 2]. Moreover, rapidly progressive fatal HP cases caused by *S. maltophilia* have been reported; however, only descriptive studies have been published [10–20].

Here, in which we included *S. maltophilia* bacteremia in patients with a hematologic malignancy, HP was found to be the most important and an independent risk factor for mortality. A previous study had reported that the incidence of HP in patients with a hematologic malignancy who had undergone allogenic hematopoietic stem cell transplantation was 2.0% [15]. In our study, the proportion of HP in patients with a hematologic malignancy and *S. maltophilia* bacteremia was 16.9% (20/118), the third most common clinical presentation in our study. This finding suggests that although HP might be rare in the general population of patients with *S. maltophilia* infection, HP could be a unique clinical presentation frequently associated with patients with a hematologic malignancy.

As shown in the review of our and previous studies [10–20], patients with HP had a common set of clinical characteristics. Acute leukemia was eightfold more common in

**Table 5** Clinical characteristics of hemorrhagic pneumonia related to *Stenotrophomonas maltophilia* in reported cases

| Variable | Hemorrhagic pneumonia (54) |
|---|---|
| Age (mean ± SD) | 49.1 ± 18.9 |
| Male | 32 (59.3) |
| Hematologic malignancy | 52 (96.3) |
| Acute leukemia | 40/52 (74.1) |
| ALL | 4/40 |
| AML | 35/40 |
| Aggressive NK/T cell leukemia | 1/40 |
| CML-BP | 2/52 (3.8) |
| MDS | 4/52 (7.7) |
| NHL | 5/52 (9.6) |
| MF | 1/52 (2.0) |
| Treatment and prophylaxis | |
| Allo-HSCT | 21/52 (40.3) |
| Recurrent or refractory malignancy | 22/52 (42.3) |
| TMP/SMX prophylaxis | 5/52 (9.6) |
| Neutropenia | 51 (94.4) |
| Bacteremia | 48 (88.9) |
| Definitive treatment | 18 (33.3) |
| TMP/SMX | 7/18 (38.9) |
| Fluoroquinolone | 2/18 (11.1) |
| Combination | 9/18 (50.0) |
| Death | 51 (94.4) |
| Time to death (median, (IQR)) | 2 (1–3) |
| 7-day mortality | 49/51 (96.1) |

Data are number (%) of patients

*SD*, standard deviation; *ALL*, acute lymphoblastic leukemia; *AML*, acute myeloid leukemia; *CML-BP*, chronic myelogenous leukemia in blastic phase; *MDS*, myelodysplastic syndrome; *NHL*, non-Hodgkin's lymphoma; *MF*, myelofibrosis; *Allo-HSCT*, allogenic hematopoietic stem-cell transplantation; *TMP/SMX*, trimethoprim-sulfamethoxazole; *IQR*, interquartile range

patients with HP than patients with non-Hodgkin lymphoma. In addition, approximately 90% of patients had neutropenia and *S. maltophilia* bacteremia. Current guidelines recommend that all patients with acute lymphoblastic leukemia and leukemic patients who have undergone allogenic hematopoietic stem cell transplantation should take antimicrobial prophylaxis for *Pneumocystis jirovecii* and TMP/SMX is preferred regimen [35]. However, the proportion of patients that received TMP/SMX prophylaxis was considerably lower than the proportion of patients that needed the prophylaxis. Mortality was extremely high in most cases reported, especially in patients with a hematologic malignancy. These finding—the facts that leukemic patients were vulnerable to HP and HP cases showed extremely high mortality—can explain why a previous study had shown that the mortality in cancer patients with *S. maltophilia* pneumonia was independently associated with hematologic malignancy and not solid cancers [36].

There have been no clear pathophysiological mechanisms underlying HP or other clinical manifestations related to *S. maltophilia* proposed. There was an in vitro study showing that protease *StmPr1* induces programmed cell death of lung epithelial cells by detaching epithelial cell from the surrounding extracellular matrix (anoikis) and by stimulating cytokine secretion from lung epithelial cells [23]. A prior study from the clinic has reported that *S. maltophilia* isolates of patients with cystic fibrosis (CF) are less effective biofilm producers than isolates from non-CF patients [22]. In our study, no significant difference in virulence and biofilm formation was observed between isolates from HP and CRBSI cases. However, isolates from HP cases seemed to have more protease activity than isolates from CRBSI cases. Clinically, thrombocytopenia and neutropenia were frequently observed in HP patients. The protease activity level of *S. maltophilia* and thrombocytopenia may make patients more prone to HP.

For improving the survival of HP cases, the early and proper use of antibiotics has been emphasized [10–20], and the new identification method, such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), helps physician to prescribe timed and appropriate antibiotics. However, administration of the appropriate antibiotics is still difficult due to the rapid progression and lethality of HP. Therefore, we should not only emphasize early administration of proper antibiotics, but also prevention of *S. maltophilia* infection. Hospital tap water and environmental sites that can be contaminated by water, such as sinks, faucets, and hemodialysis units, could be sources of outbreak for *S. maltophilia* infection [37]. In our previous studies, *S. maltophilia* colonization before bacteremia and lack of TMP/SMX prophylaxis were predictive factors of *S. maltophilia* bacteremia among adult patients with a hematologic malignancy and gram-negative bacteremia [25]. There is no specific strategy for prevention of *S. maltophilia* transmission; however, we should implement general infection control strategies: barrier protection, surveillance of *S. maltophilia* isolation, extensive cleaning of sites that can be contaminated by tap water, and measures against ventilator-associated pneumonia. Furthermore, given that *S. maltophilia* colonization may be an important step in developing bacteremia and a lack of TMP/SMX prophylaxis may be related to *S. maltophilia* infection, the strategy of prophylactic TMP/SMX administration to prevent *S. maltophilia* infection should be evaluated.

There are several limitations in our study. This study was implemented retrospectively in a single medical center. In addition, HP was defined by a patient's clinical symptoms. However, our HP cases showed very similar clinical features as compared to previous case studies of HP. Because of the limited number of cases, we could not find any statistical significance in microbiological analysis between HP and CRBSI cases. Despite these limitations, to our knowledge, this

is the first large study to evaluate HP as an independent risk factor for mortality and to compare clinical and microbiological characteristics between HP and CRBSI cases caused by *S. maltophilia*.

In conclusion, overall mortality of *S. maltophilia* bacteremia in patients with a hematologic malignancy is still high, and HP accounts for a large proportion of mortality of such patients. HP is a relatively common clinical manifestation of *S. maltophilia* bacteremia in patients with a hematologic malignancy. Despite evidence of microbiological is weak, it seems that the unique characteristics of patients and microbes contribute to the pathogenesis of these representative clinical manifestations. Given the rapidly fatal clinical course of HP, prevention should be emphasized as well as appropriate treatment.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical statement** The study was approved by the local ethical research committee (IRB number: 2017-08-016-002).

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