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Original Article

Pseudomonas aeruginosa Bacteremia among Immunocompetent and Immunocompromised Patients: Relation to Initial Antibiotic Therapy and Survival

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SUMMARY: *Pseudomonas aeruginosa* bacteremia occurs mainly in immunocompromised patients. However, *P. aeruginosa* bacteremia in immunocompetent patients has also been reported. The aim of this study was to evaluate the clinical characteristics of *P. aeruginosa* bacteremia in relation to the immune status of the patients. The medical records of 126 adult patients with *P. aeruginosa* bacteremia in Nagasaki University Hospital were retrospectively reviewed between January 2003 and December 2012. Of 126 patients with *P. aeruginosa* bacteremia, 60 patients (47.6%) were classified as immunocompetent. Mortality in immunocompetent patients tended to be lower than in immunocompromised patients (7-day mortality, 8% vs. 30%, $P < 0.01$; 30-day mortality, 23% vs. 39%, $P = 0.053$). Multivariate analysis showed that a higher sequential organ failure assessment score (hazard ratio [HR]: 1.27, $P < 0.01$) and underlying malignancies (HR: 3.33, $P < 0.01$) were independently associated with 30-day mortality. Initial antibiotic therapy (HR: 0.21, $P < 0.01$) and patients' immune status (HR: 0.29, $P = 0.02$) also had a significant impact on survival. However, there was a significant interaction between these 2 variables ($P = 0.03$ for interaction). A subgroup analysis showed that in immunocompromised, but not immunocompetent patients, initial appropriate antibiotic therapy was associated with lower mortality (30-day mortality 20.5% vs. 66.7%, $P < 0.01$ by log-rank test).

INTRODUCTION

Pseudomonas aeruginosa is an important gram-negative pathogen and can cause a wide range of acute and chronic infectious diseases. The major sites of infection are the respiratory tract, urinary tract, abdomen, and wounds. *P. aeruginosa* can also invade the bloodstream from these sites, resulting in a systemic infection called bacteremia (1). *P. aeruginosa* bacteremia often progresses to a serious condition and has a poor prognosis with a reported mortality rate of approximately 26% to 39% (2–4).

P. aeruginosa has long been recognized as an opportunistic pathogen that usually causes infection in immunocompromised patients (3,5). However, there are several reports of severe *P. aeruginosa* infection in patients without apparent immunodeficiency (6–9). Some cases of *P. aeruginosa* community-acquired pneumonia described in these reports were caused by inhalation of contaminated water aerosols. Such cases were frequently accompanied by bacteremia and had as high a mortality risk as the immunocompromised patients with bacteremia (7). However, little is known about the clinical differences, such as age, sex, source of infection, or the effect of antibiotic therapy between immunocompetent and immunocompromised patients with *P. aeruginosa* bacteremia.

The type III secretion system (TTSS) is one of the most important virulence factors of *P. aeruginosa* (10). Through the TTSS, *P. aeruginosa* transfers effector proteins from the bacterial cytoplasm directly into the host cell. Four effector proteins of *P. aeruginosa* have been characterized so far: ExoS, ExoT, ExoU, and ExoY. The prevalence of the genes encoding these virulence proteins is varied among *P. aeruginosa* isolated from different sources or clinical conditions (11,12).

The aim of this study was to evaluate the clinical and microbiological characteristics including TTSS genotype of *P. aeruginosa* bacteremia in relation to the immune status of the patients.

MATERIALS AND METHODS

Study design and population: A retrospective cohort study was conducted at Nagasaki University Hospital, an 862-bed tertiary care teaching hospital in Nagasaki, Japan. All patients aged 18 years and older with at least one positive blood culture for *P. aeruginosa* identified between January 2003 and December 2012 were included. A total of 126 patients were identified through an electronic database of the clinical microbiology unit. This study and the waiver of informed consent were approved by the institutional ethics committee of

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Nagasaki University Hospital (approval no. 13022527).

Data collection and definitions: Patients' medical records were reviewed to collect the following clinical data: demographic characteristics, duration of hospital stay, comorbidities, vital signs, laboratory results, severity of diseases, prior surgery or antibiotic therapy, microbiological data, and mortality data. Severity of disease was assessed by the sequential organ failure assessment (SOFA) score (13). The primary source of bacteremia was determined according to U.S. Centers for Disease Control and Prevention criteria (14). The initial antibiotic therapy was considered appropriate when it was administered at an optimal dosage within 48 h after the acquisition of a blood culture sample and included at least one antibiotic that was active in vitro against *P. aeruginosa* isolated in the blood culture (15). Because the definition of immunosuppression varies among studies, we defined severely immunocompromised patients according to previous studies of *P. aeruginosa* bacteremia (4,16,17) as those who were receiving high-dose steroid (prednisone or equivalent $\geq$20 mg/day for at least 2 weeks) or immunosuppressive drugs, had undergone transplantation, had human immunodeficiency virus infection, had neutropenia (neutrophil count $<$ 500 cells/μl), or had undergone chemotherapy or radiotherapy within 30 days of the onset of bacteremia.

Microbiological analysis: Of the stored isolates from these patients, 112 strains of *P. aeruginosa* were available for analysis. All strains were identified using the Vitek2 system (bioMerieux, Hazelwood, MO, USA) or the Phoenix 100 instrument (BD, Franklin Lakes, NJ, USA). Antibiotic susceptibility of the isolates was tested by a broth dilution method or an automatic method and determined according to the Clinical and Laboratory Standards Institute interpretive criteria (18). Isolates with intermediate susceptibility were considered to be resistant. Sitafloxacin resistance was defined as a minimum inhibitory concentration of $\geq$2 μg/ml.

The genotype of TTSS was determined by multiplex polymerase chain reaction (PCR) assays. All strains were grown overnight in Luria-Bertani broth at 37°C with shaking. Chromosomal DNA was extracted from *P. aeruginosa* using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. TTSS genes (*exoS*, *exoU*, *exoY*, *exoT*, and *pcrV*) in isolated strains were analyzed by PCR using primers and conditions previously described (19,20). *P. aeruginosa* strains PAO1 ($exoS^+/exoT^+/exoY^+$) and PA103 ($exoU^+/exoT^+/exoY^+$) were used as positive controls.

Statistics: The data are described descriptively using medians and interquartile ranges for continuous variables and percentages for categorical variables. Categorical variables were evaluated using the χ^2 or Fisher's exact test, and continuous variables were evaluated using Mann-Whitney U tests, as appropriate. Risk factors associated with 30-day mortality were evaluated by Cox proportional hazards models using univariate analysis followed by multivariate analysis. Factors with a *P*-value $<$ 0.1 in univariate analysis were included in the multivariate analysis. The variables were checked for multicollinearity using the variance inflation factor (VIF) and excluded from the model if the VIF exceeded 2. To assess whether the effect of initial antibiotic therapy was modified by the patient's immune status or other factors, interactions terms between appropriate antibiotic therapy and covariates were also included in the model. Backward stepwise selection was used to choose the variables for the multivariate Cox regression model. The proportional hazards assumption was checked using log-minus-log curves for categorical variables and time-dependent covariates for continuous variables. No violation of the assumption was detected. Patient survival curves were plotted by using the Kaplan-Meier method and compared using the log-rank test. All statistical analyses were performed using SPSS version 21.0 statistical software (SPSS, Inc., Chicago, IL, USA).

RESULTS

Clinical features and outcomes: Among the 126 patients with *P. aeruginosa* bacteremia, 60 patients (47.6%) were classified as immunocompetent. The clinical characteristic of patients are shown in Table 1. Immunocompetent patients were more likely to be older ($P < 0.01$), to have had a shorter hospital stay before the onset of the *P. aeruginosa* bacteremia ($P = 0.047$), and to have a higher frequency of cardiovascular disorders ($P < 0.01$) and neurological disorders ($P < 0.01$) compared with immunocompromised patients. Almost half of the immunocompromised patients had a central venous catheter. During the 30 days before bacteremia, immunocompetent patients were less likely to receive antibiotic therapy with β-lactams ($P = 0.02$) or fluoroquinolones ($P = 0.01$) and more likely to undergo invasive hepatobiliary procedures ($P < 0.01$) such as endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC) than immunocompromised patients. At the onset of the bacteremia, the SOFA score was significantly lower in immunocompetent patients ($P < 0.01$). Immunocompromised patients showed low levels of white blood cells ($P < 0.01$), neutrophils ($P < 0.01$), and platelets ($P < 0.01$). The common sources of bacteremia were urinary tract (30.0%) and intra-abdominal (28%) infections in the immunocompetent patients, whereas the source was unknown in almost one-third of the immunocompromised patients. Mortality in the immunocompetent patients tended to be lower than in the immunocompromised patients (7-day mortality, 8% vs. 30%, $P < 0.01$; 30-day mortality, 23% vs. 39%, $P = 0.053$).

Microbiological features of *P. aeruginosa* strains: As shown in Table 2, 3 TTSS genes (*exoY*, *exoT*, and *pcrV*) were present in almost all strains. The majority (77.7%) of *P. aeruginosa* strains harbored the *exoS* gene, whereas only a few strains (17.0%) harbored the *exoU* gene. The prevalence of these genes was not different between isolates from immunocompetent patients and immunocompromised patients. *P. aeruginosa* strains were classified into 4 groups according to the presence of either the *exoS* or the *exoU* gene. The $exoS^+/exoU^-$ genotype (75.9%) was the most prevalent, followed by $exoS^-/exoU^+$ (15.2%), $exoS^-/exoU^-$ (7.1%), and $exoS^+/exoU^+$ (1.8%). Although not significant, the $exoS^-/exoU^-$ genotype tended to be more frequent in isolates from immunocompromised

Table 1. Clinical characteristics of patients with *Pseudomonas aeruginosa* bacteremia

Variable	Immunocompetent (n = 60)	Immunocompromised (n = 66)	P-value
Age, median yr (range)	69 (62–78)	62 (55–72)	<0.01
Gender			0.13
Male	44 (73%)	40 (61%)	
Female	16 (27%)	26 (39%)	
Immunosuppressive states and medications			
Receiving steroids or immunosuppressive drugs	—	42 (64%)	
Post-transplantation	—	12 (18%)	
Neutropenia	—	22/55 (40%)	
Recent chemotherapy or radiotherapy	—	26 (39%)	
Hospital stay, median days (IQR)			
Before onset of bacteremia	19 (6–50)	29 (13–74)	0.047
Comorbidities			
Malignant diseases	25 (42%)	34 (52%)	0.27
Cardiovascular disorders	15 (25%)	5 (8%)	<0.01
Neurological disorders	15 (25%)	2 (3%)	<0.01
Presence of invasive devices			
Central venous catheter	17 (28%)	35 (53%)	<0.01
Events within 30 days before onset of bacteremia			
β-Lactam usage	21 (35%)	37 (56%)	0.02
Fluoroquinolone usage	9 (15%)	23 (35%)	0.01
Invasive hepatobiliary procedures[1)]	10 (17%)	1 (2%)	<0.01
Severity parameter, median (IQR)			
SOFA score	4 (3–7)	6 (4–10)	<0.01
Laboratory data, median (IQR)			
WBC (/μl)	11,600 (7,500–15,600)	3,300 (900–10,200)	<0.01
ANC (/μl)	10,400 (5,990–14,600)	1,300 (70–8,700)	<0.01
Platelets (10^4/μl)	14.9 (10.9–25.1)	4.8 (2.3–10.8)	<0.01
Source of bacteremia			<0.01
Respiratory	6 (10%)	14 (21%)	
Urinary	18 (30%)	7 (11%)	
Abdominal[2)]	17 (28%)	15 (23%)	
Other	10 (17%)	6 (9%)	
Unknown	9 (15%)	24 (36%)	0.63
Initial antibiotic therapy			
Appropriate	38 (63%)	39 (59%)	
Inappropriate	22 (37%)	27 (41%)	
Overall mortality			
7-day mortality	5 (8%)	20 (30%)	<0.01
30-day mortality	14 (23%)	26 (39%)	0.053

Data are expressed as numbers (percentages) except if indicated otherwise.
1): ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiography.
2): Hepatobiliary tract and gastrointestinal tract.
HIV, human immunodeficiency virus; SOFA, sequential organ failure assessment; IQR, interquartile range; WBC, white blood cell; ANC, absolute neutrophil count; CRP, c-reactive protein.

patients than in those from immunocompetent patients (12% vs. 2%, $P = 0.064$).

The results of the antibiotic susceptibility tests are shown in Table 3. The strains isolated from immunocompetent patients showed lower rates of resistance to β-lactams and fluoroquinolones than isolates from immunocompromised patients. Aminoglycosides showed good susceptibility against both isolated strains.

Risk factors associated with mortality: Table 4 shows the univariate and multivariate analyses of the risk factors for mortality among all patients with *P. aeruginosa* bacteremia. In a univariate Cox proportional hazards model, factors significantly associated with 30-day mortality were higher SOFA score (hazard ratio [HR]: 1.21, $P < 0.01$) and C-reactive protein level (HR: 1.04, $P = 0.01$), lower platelet count (HR: 1.12, $P < 0.01$), and underlying malignancies (HR: 1.96, $P = 0.04$). Appropriate initial antibiotic therapy (HR: 0.31, $P < 0.01$) reduced the risk of death within 30 days. TTSS genotypes did not correlate with clinical outcome.

The results of multivariate analysis showed that higher SOFA score (HR: 1.27, $P < 0.01$), underlying malignancies (HR: 3.33, $P < 0.01$), appropriate initial antibiotic therapy (HR: 0.21, $P < 0.01$), and immunocompetent status (HR: 0.29, $P = 0.02$) were independent risk factors for mortality. Although immunocompetent status and appropriate initial antibiotic therapy decreased mortality risk in multivariate analysis, there were significant interaction effects between these variables ($P = 0.03$ for interaction). Hence, the patients were

Table 2. Prevalence of TTSS genes in *Pseudomonas aeruginosa* clinical isolates

Variable	Immunocompetent (n = 53)	Immunocompromised (n = 59)	P-value
TTSS genes			
exoY	51 (96%)	58 (98%)	0.49
exoT	53 (100%)	59 (100%)	—
exoS	42 (79%)	45 (76%)	0.71
exoU	12 (23%)	7 (12%)	0.13
pcrV	53 (100%)	59 (100%)	—
TTSS genotypes			0.064
exoS−/*exoU*−	1 (2%)	7 (12%)	
exoS+/*exoU*−	40 (75%)	45 (76%)	
exoS−/*exoU*+	10 (19%)	7 (12%)	
exoS+/*exoU*+	2 (4%)	0	

Data are expressed as numbers (percentages) except if indicated otherwise.
TTSS, type III secretion system.

Table 3. Antibiotic resistance patterns of *Pseudomonas aeruginosa* clinical isolates

Variable	Immunocompetent (n = 53)	Immunocompromised (n = 59)	P-value
Resistance to antibiotics			
Tazobactam/ Piperacillin	6 (11%)	22 (37%)	<0.01
Ceftazidime	10 (19%)	23 (39%)	0.02
Cefepime	10 (19%)	20 (34%)	0.07
Aztreonam	23 (43%)	35 (59%)	0.09
Imipenem	17 (32%)	24 (41%)	0.35
Meropenem	10 (19%)	20 (34%)	0.07
Ciprofloxacin	8 (15%)	25 (42%)	<0.01
Levofloxacin	8 (15%)	26 (44%)	<0.01
Sitafloxacin	4 (8%)	17 (29%)	<0.01
Gentamicin	5 (9%)	11 (19%)	0.16
Tobramycin	2 (4%)	9 (15%)	0.04

Data are expressed as numbers (percentages) except if indicated otherwise.

Table 4. Risk factors for mortality in patients with *Pseudomonas aeruginosa* bacteremia

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
SOFA score (per point increase)	1.21	1.13–1.28	<0.01	1.27	1.17–1.38	<0.01
CRP (per mg/dl increase)	1.04	1.01–1.08	0.013			
Platelets (per $10^4/\mu$l decrease)	1.12	1.06–1.18	<0.01			
Malignant diseases	1.96	1.04–3.69	0.04	3.33	1.56–7.1	<0.01
Appropriate initial antibiotic therapy	0.31	0.17–0.59	<0.01	0.21	0.09–0.51	<0.01
Immunocompetent state	0.53	0.28–1.01	0.055	0.29	0.11–0.8	0.02
TTSS genotypes[1)]						
exoS−/*exoU*−	1.0					
exoS+/*exoU*−	3.14	0.43–23.1	0.26			
exoS−/*exoU*+	2.76	0.32–23.7	0.35			

[1)]: Strains with *exoS*+/*exoU*+ genotype were excluded because there were no events.
CI, confidence interval; SOFA, sequential organ failure assessment; CRP, c-reactive protein; TTSS, type III secretion system.

divided into subgroups according to their immune status, and the effect of initial antibiotic therapy on outcome was evaluated using Kaplan-Meier survival curves. A subgroup analysis showed that initial appropriate antibiotic therapy was associated with lower mortality in immunocompromised patients (30-day mortality 20.5% vs. 66.7%, $P < 0.01$ by log-rank test), but not in immunocompetent patients (30-day mortality 21.1% vs. 27.3%, $P = 0.48$ by log-rank test) (Fig. 1).

DISCUSSION

Our study revealed that immunocompetent patients had a lower SOFA score and early mortality rate compared with immunocompromised patients, although the outcome of both groups was poor. *P. aeruginosa* strains isolated from immunocompetent patients were more often sensitive to antibiotics than those isolated from immunocompromised patients. Importantly, the effects of initial antibiotic therapy on outcome were different depending on the immune status of the patients. In many previous reports, *P. aeruginosa* bacteremia in immunocompetent individuals was associated with pneumonia that occurred after exposure to aerosols of contaminated water (7,9). However, in this study, the major causes of bacteremia in immunocompetent patients were intra-abdominal and urinary tract infections. Normal host defenses usually prevent binding or invasion of *P. aeruginosa* to host tissues. Therefore, infectious causes, such as a high bacterial inoculum and epithelial barrier damage, are required for *P. aeruginosa* to invade tissues and spread systemically in hosts with normal immunity (21). In fact, immunocompetent patients in this study often developed bacteremia following an anastomotic leak after gastrointestinal surgery or mucosal barrier injury that was induced by invasive procedures such as ERCP, PTC, and urinary catheterization.

The majority of *P. aeruginosa* strains were shown to harbor both *exoT* and *exoY* genes, whereas the presence of *exoS* and *exoU* genes was generally mutually exclusive (11). The results of the present study were consistent with these findings. The strains that possessed neither the *exoS* nor the *exoU* gene (*exoS*$^-$/*exoU*$^-$) were isolated almost exclusively from immunocompromised patients. The *exoS*$^-$/*exoU*$^-$ strains have been shown to have low virulence (12) and therefore may be able to cause bacteremia mainly in patients with an im-

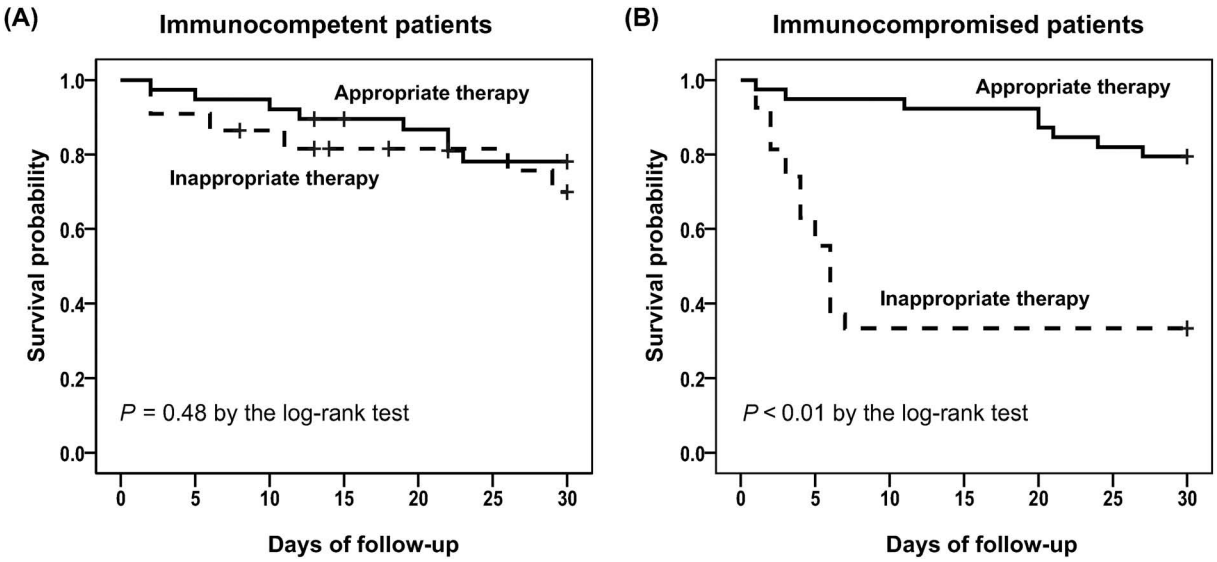

Fig. 1. Kaplan-Meier curves of 30-day survival of immunocompetent patients (A) or immunocompromised patients (B) are stratified by appropriateness of initial antibiotic therapy. Cross marks indicate censored data.

munocompromised status.

The resistance profiles to antibiotics also greatly differed between the 2 groups. Many studies have shown a correlation between antibiotics use and subsequent resistance to antibiotics (22). Because the immunocompromised patients had more frequently received previous antibiotic treatment with β-lactam or fluoroquinolone in the present study, the risk for the development of antibiotic resistance may increase in these patients.

The overall 30-day mortality (32%) in our study is similar to that in previous studies (2–4). Previously reported risk factors for mortality in patients with *P. aeruginosa* bacteremia include age, severity of illness (acute physiology and chronic health evaluation and SOFA scores), underlying comorbidities (malignancy, liver disease, and renal failure), and initial site of infection (23–25). Our results showed significant associations of a higher SOFA score and malignancy with mortality. Initial antibiotic therapy and patients' immune status also had a significant impact on patient survival. However, there was a significant interaction between these 2 variables. Appropriate initial antibiotic therapy is thought to improve the outcome of patients with bacteremia in general (26). In cases of *P. aeruginosa* bacteremia, however, the effectiveness of initial antibiotic therapy remains controversial, as some studies report no impact of initial antibiotic therapy on outcomes (27). This may be at least partly because of the variation in patient characteristics between these studies, and therefore, it is possible that there might be certain factors that affect the efficacy of initial antibiotic therapy. In a prospective analysis, Schechner et al. evaluated 76 patients with *P. aeruginosa* bacteremia and found that appropriate initial antibiotic therapy improved survival only in patients who had severe sepsis or septic shock (28). We performed a subgroup analysis using the Kaplan-Meier method, and the log-rank test showed a significant influence of initial antibiotic therapy in immunocompromised patients, whereas it did not show any therapeutic benefit in immunocompetent patients. Similar findings that support our results were observed in neutropenic and non-neutropenic patients with bacteremia (29,30). Therefore, considering these observations, initial antibiotic therapy for *P. aeruginosa* bacteremia should be highlighted in patients with immunocompromised status. Because of the high resistance rates of the isolates from immunocompromised patients to antibiotics except aminoglycosides in our study, combination therapy, especially with aminoglycosides, is expected to provide better initial coverage and outcomes for these patients, as described previously in patients with severe gram-negative infections (31).

The present study has several limitations, including a relatively small number of patients in a single tertiary hospital. This study was based on retrospective data collected from medical records, and because documentation may have been incomplete or missing, there was a potential risk of bias.

In conclusion, our study demonstrated considerable differences in the clinical and microbiological features of immunocompetent and immunocompromised patients with *P. aeruginosa* bacteremia. Appropriate initial antibiotic therapy is an important prognostic factor, especially for immunocompromised patients. Further studies are needed to develop proper treatment for *P. aeruginosa* bacteremia according to the patient's status.

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Conflict of interest None to declare.

REFRENCES

1. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005;41:848-54.
2. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309-17.

3. Al-Hasan MN, Wilson JW, Lahr BD, et al. Incidence of *Pseudomonas aeruginosa* bacteremia: a population-based study. Am J Med. 2008;121:702-8.
4. Osmon S, Ward S, Fraser VJ, et al. Hospital mortality for patients with bacteremia due to *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Chest. 2004;125:607-16.
5. Morrison AJ, Wenzel RP. Epidemiology of infections due to *Pseudomonas aeruginosa*. Rev Infect Dis. 1984;6 Suppl 3: S627-42.
6. Henderson A, Kelly W, Wright M. Fulminant primary *Pseudomonas aeruginosa* pneumonia and septicaemia in previously well adults. Intensive Care Med. 1992;18:430-2.
7. Hatchette TF, Gupta R, Marrie TJ. *Pseudomonas aeruginosa* community-acquired pneumonia in previously healthy adults: case report and review of the literature. Clin Infect Dis. 2000;31: 1349-56.
8. Huang YC, Lin TY, Wang CH. Community-acquired *Pseudomonas aeruginosa* sepsis in previously healthy infants and children: analysis of forty-three episodes. Pediatr Infect Dis J. 2002;21: 1049-52.
9. Huhulescu S, Simon M, Lubnow M, et al. Fatal *Pseudomonas aeruginosa* pneumonia in a previously healthy woman was most likely associated with a contaminated hot tub. Infection. 2011;39: 265-9.
10. Hauser AR. The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. Nat Rev Microbiol. 2009;7:654-65.
11. Feltman H, Schulert G, Khan S, et al. Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. Microbiology. 2001;147:2659-69.
12. Berthelot P, Attree I, Plésiat P, et al. Genotypic and phenotypic analysis of type III secretion system in a cohort of *Pseudomonas aeruginosa* bacteremia isolates: evidence for a possible association between O serotypes and exo genes. J Infect Dis. 2003;188: 512-8.
13. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on ''sepsis-related problems'' of the European Society of Intensive Care Medicine. Crit Care Med. 1998;26: 1793-800.
14. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36:309-32.
15. Micek ST, Lloyd AE, Ritchie DJ, et al. *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother. 2005;49: 1306-11.
16. Schechner V, Nobre V, Kaye KS, et al. Gram-negative bacteremia upon hospital admission: when should *Pseudomonas aeruginosa* be suspected? Clin Infect Dis. 2009;48:580-6.
17. Peña C, Suarez C, Ocampo-Sosa A, et al. Effect of adequate single-drug vs combination antimicrobial therapy on mortality in *Pseudomonas aeruginosa* bloodstream infections: a post Hoc analysis of a prospective cohort. Clin Infect Dis. 2013;57:208-16.
18. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 22th informational supplement. Document M100-S22. Wayne, PA: CLSI; 2012.
19. Ajayi T, Allmond LR, Sawa T, et al. Single-nucleotide-polymorphism mapping of the *Pseudomonas aeruginosa* type III secretion toxins for development of a diagnostic multiplex PCR system. J Clin Microbiol. 2003;41:3526-31.
20. Wong-Beringer A, Wiener-Kronish J, Lynch S, et al. Comparison of type III secretion system virulence among fluoroquinolone-susceptible and -resistant clinical isolates of *Pseudomonas aeruginosa*. Clin Microbiol Infect. 2008;14:330-6.
21. Engel J, Eran Y. Subversion of mucosal barrier polarity by *Pseudomonas aeruginosa*. Front Microbiol. 2011;2:114.
22. Gbaguidi-Haore H, Dumartin C, L'Hériteau F, et al. Antibiotics involved in the occurrence of antibiotic-resistant bacteria: a nationwide multilevel study suggests differences within antibiotic classes. J Antimicrob Chemother. 2013;68:461-70.
23. Lodise TP Jr, Patel N, Kwa A, et al. Predictors of 30-day mortality among patients with *Pseudomonas aeruginosa* bloodstream infections: impact of delayed appropriate antibiotic selection. Antimicrob Agents Chemother. 2007;51:3510-5.
24. Scheetz MH, Hoffman M, Bolon MK, et al. Morbidity associated with *Pseudomonas aeruginosa* bloodstream infections. Diagn Microbiol Infect Dis. 2009;64:311-9.
25. Parkins MD, Gregson DB, Pitout JD, et al. Population-based study of the epidemiology and the risk factors for *Pseudomonas aeruginosa* bloodstream infection. Infection. 2010;38:25-32.
26. Leibovici L, Shraga I, Drucker M, et al. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J Intern Med. 1998;244:379-86.
27. Osih RB, McGregor JC, Rich SE, et al. Impact of empiric antibiotic therapy on outcomes in patients with *Pseudomonas aeruginosa* bacteremia. Antimicrob Agents Chemother. 2007;51: 839-44.
28. Schechner V, Gottesman T, Schwartz O, et al. *Pseudomonas aeruginosa* bacteremia upon hospital admission: risk factors for mortality and influence of inadequate empirical antimicrobial therapy. Diagn Microbiol Infect Dis. 2011;71:38-45.
29. Kang CI, Kim SH, Kim HB, et al. *Pseudomonas aeruginosa* bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clin Infect Dis. 2003;37:745-51.
30. Lin MY, Weinstein RA, Hota B. Delay of active antimicrobial therapy and mortality among patients with bacteremia: impact of severe neutropenia. Antimicrob Agents Chemother. 2008;52: 3188-94.
31. Micek ST, Welch EC, Khan J, et al. Empiric combination antibiotic therapy is associated with improved outcome against sepsis due to Gram-negative bacteria: a retrospective analysis. Antimicrob Agents Chemother. 2010;54:1742-8.