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CLINICAL OUTCOMES OF TYPE III PSEUDOMONAS AERUGINOSA BACTEREMIA

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Abstract

Background—*Pseudomonas aeruginosa* bacteremia is a serious and life-threatening infection associated with high mortality. Among the multitude of virulence determinants possessed by *P. aeruginosa*, the type III secretion system (TTSS) has been implicated with more acute and invasive infection in respiratory diseases. However, the relationship between TTSS and clinical outcomes in *P. aeruginosa* bacteremia has not been investigated.

Objectives—To determine the association between the TTSS virulence factor in *P. aeruginosa* blood stream infection and 30-day mortality.

Design—Retrospective analysis of 85 cases of *P. aeruginosa* bacteremia.

Setting—Tertiary care hospital.

Interventions—Bacterial isolates were assayed in vitro for secretion of type III exotoxins (ExoU, ExoT, and ExoS). Strain-relatedness was analyzed using randomly amplified polymorphic DNA PCR genotyping. Antimicrobial susceptibilities were determined by means of the Kirby-Bauer disk-diffusion test.

Measurements and Main results—At least one of the TTSS proteins was detected in 37 out of the 85 isolates (44%). Septic shock was identified in 43% of bacteremic patients with TTSS+ isolates compared to 23% of patients with TTSS− isolates ($p=0.12$). A high frequency of resistance in the TTSS+ isolates was observed to ciprofloxacin (59%), cefipime (35%), and gentamycin (38%). There was a significant difference in the 30-day cumulative probability of

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death after bacteremia between secretors and nonsecretors ($p=0.02$). None of the TTSS+ patients who survived the first 30 days had a *P. aeruginosa* isolate which exhibited ExoU phenotype.

Conclusions—The expression of TTSS exotoxins in bacteremic isolates of *P. aeruginosa* confers poor clinical outcomes independent of antibiotic susceptibility profile.

Keywords

Type III secretion system; blood stream infection; *Pseudomonas*; shock; mortality; antimicrobial resistance

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous aerobic Gram-negative organism that rarely causes disease in healthy individuals. However, *Pseudomonas aeruginosa* is a common cause of infection in hospitalized patients accounting for 11–14% of all nosocomial infections (1–3), including pneumonias, urinary tract infections, and postoperative wound infections. *P. aeruginosa* ranks third after *Escherichia coli* and *Klebsiella* species among Gram negative bacteria isolated during nosocomial blood stream infections and seventh among all pathogens (1). Despite improvements in hospital care, the prognosis of *P. aeruginosa* bacteremia remains poor with a crude mortality ranging between 33% to 61% (4, 5).

The identification of prognostic factors, potentially influencing the mortality of *P. aeruginosa* bacteremia, is essential in order to decide whether a subgroup of patients might deserve a particularly aggressive therapeutic approach. Evidence derived from clinical investigations indicates that septic shock, initial site of infection, and immunosuppression at the time of *P. aeruginosa* bacteremia are considered risk factors for mortality (6–8). A key determinant of *P. aeruginosa* pathogenicity is its ability to deliver a number of toxins into host cells via a type III secretion system (TTSS) (9). The system uses a complex secretion and translocation machinery to inject a set of exotoxins directly into the cytoplasm of eukaryotic cells. Four effector proteins have been identified: ExoU, a phospholipase; ExoY, an adenylate cyclase; and ExoT and ExoS, which are bifunctional proteins (10, 11). Although all strains harbor type III protein secretory genes, only few of these are capable of secreting effector proteins (12). The expression of the type III secretion system in *P. aeruginosa* isolates has been associated with higher bacterial burden and increased mortality in patients with lower respiratory tract infections (13, 14). To date, no study to our knowledge has investigated the impact of type III phenotypic expression on clinical outcomes of *P. aeruginosa* bloodstream infection. We hypothesized that the production of type III secretory proteins is associated with poor outcomes in *P. aeruginosa* bacteremia.

MATERIALS AND METHODS

Study population

The study was conducted at the VA Western New York Healthcare System, a tertiary care facility affiliated with the State University of New York at Buffalo following approval by the Institutional Review Board. A retrospective cohort study was established based on *P. aeruginosa* blood isolates that were stored in a databank from a city wide surveillance initiative implemented in 2004. All corresponding adult patients admitted to the study hospital between January 2004 and June 2010 were identified from the Computerized Patient Record System. Patients with polymicrobial bloodstream infection defined as the isolation of 2 or more different organisms from the same blood culture were excluded. Patients were included only once in the database to ensure independence of observation. Among those with multiple bacteremic episodes, only the first event was analyzed.

The cohort database included information on sociodemographic characteristics (age, gender, race, and residence), comorbidities, prior antibiotic therapy, severity of illness, source of bacteremia according to the Centers for Disease Control (CDC) and Prevention criteria (15), choice and dose of antibiotics used, time to first dose of antimicrobial therapy, use of corticosteroids and other immunosuppressive agent.

Bacterial isolates

P. aeruginosa clinical specimens, including the reference strains PA01 and PA103, were streaked onto trypticase soy agar plates and grown in a deferrated dialysate of trypticase soy broth supplemented with 10 mM nitrilotriacetic acid (Sigma Chemicals), 1% glycerol and 100 mM monosodium glutamate at 37°C for 17 h in a shaking incubator at 250 rpm. Bacteria were removed by centrifugation at 8500 x g for 5 min, after which the supernatant was collected and concentrated 20-fold by the addition of a saturated solution of ammonium sulfate.

Immunoblot analysis

Standardized protein concentrations for the cell-free supernatant were loaded onto 12.5% Tris polyacrylamide gels (Biorad; Hercules, CA) and run under denaturing conditions. Polyacrylamide gels were transferred to PVDF membrane and immunoblotted with polyclonal rabbit antiserum against ExoU, ExoT, and ExoS as previously described (16).

PCR genotyping

The clonality of *P. aeruginosa* isolates was determined using randomly amplified polymorphic DNA (RAPD) PCR genotyping as previously described (16).

Susceptibility profile

Antimicrobial susceptibilities of *P. aeruginosa* isolates were determined by means of the Kirby-Bauer disk-diffusion test on Mueller-Hinton agar (BBL Microbiologic System, Cockeysville, MD) for imipenem, piperacillin-tazobactam, cefipime, gentamycin, amikacin, and ciprofloxacin. Minimum inhibitory concentration (MIC) breakpoints and quality-control protocols were used according to standards established by the Clinical and Laboratory Standards Institute (17). Isolates with intermediate susceptibility were classified as resistant for analysis.

Definitions

Isolates that secreted type III proteins (ExoU, ExoS, ExoT) were referred to as type III secretion positive (TTSS+) isolates, and isolates that did not secrete these proteins were referred to as type III secretion negative (TTSS-). *P. aeruginosa* bacteremia met the CDC criteria for infection (15). Shock was defined as a decrease in systolic blood pressure to a level of less than 90 mm Hg or a decrease of at least 40 mm Hg below baseline blood pressure, despite adequate fluid resuscitation, in conjunction with organ dysfunction and perfusion abnormalities (18). Severity of illness was determined in accordance with the Acute Physiologic and Chronic Health Evaluation (APACHE) II (19). The burden of comorbidities was assessed by the Charlson Index (20).

All patients with septic shock were treated according to a standardized protocol that combined early goal-directed therapy, prompt initiation and appropriate dosing of antimicrobial therapy, hydrocortisone supplementation in stress doses, and an evaluation for drotrecogin alfa infusion. Antimicrobial therapy was judged to be either adequate or inadequate on the basis of the in-vitro susceptibility of an isolated organism. Patients were considered to have prior antibiotic therapy if they have received an antimicrobial agent for at

least 2 days within 30 days preceding onset of *Pseudomonas* bacteremia. Recurrent *P. aeruginosa* bacteremia was defined as *P. aeruginosa* bacteremia occurring within 30 days after completion of 14 days of adequate antimicrobial therapy. Reinfection was defined as the isolation of a *P. aeruginosa* strain that is genetically distinct from the initial strain isolated from blood culture. Overall mortality and invasive ventilation-free days was calculated in the first 30 days after the first positive blood culture. Survival of patients with multiple episodes of bacteremia was assessed at day 30 from the onset of bacteremia irrespective of the results of subsequent blood cultures.

Statistical analysis

All analyses of patients were performed by investigators, without knowledge of *in vitro* susceptibility results. SPSS 15.0 (SPSS Inc., Cary, NC) was used for all statistical comparisons. The alpha level for significance was set at < 0.05 . Continuous variables were analyzed using a two-tailed student's- *t*-test for normally distributed variables and the Mann-Whitney test for non-normally distributed variables. Means and standard deviations (SDs) are presented for normal data and medians with interquartile ranges (IQRs) for non-normally distributed data. Proportions were compared using the Chi-square test with Yates correction or Fisher's exact test when necessary. The relationship between pseudomonal bacteremia and the outcome variables was investigated by a Cox proportional hazard model. All biologically plausible variables with a *p* value of < 0.20 in the bivariable analysis were considered for inclusion in the final multivariable regression models. A multicollinearity test was performed using the variance inflation factor to assess the degree of correlation between independent variables. A Kaplan-Meier product-limit method was used to estimate by univariate analysis the risk of death by presence of virulence factors in those who received adequate therapy. The cumulative probability of death across treatment groups was compared by the log-rank test.

RESULTS

Study population

During the study period, a total of 89 patients with *P. aeruginosa* bacteremia were identified. Four patients were excluded from analysis due to polymicrobial blood stream infection leaving 85 cases for analysis. Thirty seven patients (44%) had a *P. aeruginosa* isolate with at least one of the type III secretory proteins detected while 48 patients (56%) were infected with isolates that did not express any of the type III secretory proteins. Table 1 displays the clinical characteristics of the study population. There were no significant differences in age, gender, underlying comorbidities, or in primary sites of infection between patients with TTSS+ and TTSS- isolates. Similarly, vital signs and laboratory parameters of the TTSS+ and TTSS- groups were comparable at the time the blood cultures were obtained (Table 2). The median time from admission to culture positive was 2.0 days in both groups ($p=0.89$). Septic shock was identified in 43% of bacteremic patients with TTSS+ isolates compared to 23% of patients with TTSS- isolates ($p=0.12$). Although the need for vasopressors were similar in both groups, patients with TTS+ isolates required a higher dose of norepinephrine in the first 48 hours of onset of septic shock than those with TTSS- isolates ($p=0.02$). Vasopressin and hydrocortisone were also prescribed more often in the TTSS+ compared to the TTSS- group but the difference did not attain statistical significance ($p=0.08$ and $p=0.09$; respectively).

Characterization of type III secretory protein phenotype

Figure 1 displays the immunoblot analysis of the of the type III secretion phenotypes in representative samples of *P. aeruginosa* isolates. The ExoU/ExoT phenotype was the most prevalent among all TTSS+ isolates and was detected in 18 out of 37 isolates (49%) while

the ExoT/ExoS was found in 8 (22%). Of the 37 isolates, 9 (24%) expressed ExoT alone while 2 (5%) expressed ExoS with no other exotoxins. None of the isolates expressed ExoU alone. Isolates that excreted ExoU did not release ExoS and vice versa. No significant association was found between the suspected focus of infection and the ability to secrete exotoxins although a higher frequency of ExoU/ExoT was noted in isolates derived from a suspected respiratory source (Table 3).

Figure 2 depicts DNA genotyping of representative *P. aeruginosa* isolates by repetitive-element-based polymerase chain reaction assay. The corresponding RAPD profiles for the type III secretory phenotypes are shown in table 4. Overall, there were 67 different RAPD fingerprints, 53 of which contained only one strain. The other 14 RAPD fingerprints had either clonal (n=6) or closely related stains (n=8). Strain relatedness was not associated with any particular secretory phenotype or time of bacteremia onset. There was also no correlation between the presumed focus of bacteremia and RAPD grouping.

Antimicrobial therapy

Forty eight patients had previous exposure to antimicrobial therapy. Of those, 65% with TTSS+ isolates and 50% with TTSS- isolates had at least two days of antibiotics in the preceding 30 days of *P. aeruginosa* bacteremia (p=0.25). Vancomycin (39%) and ciprofloxacin (24%) were the most commonly prescribed antibiotics in the TTSS+ group while ciprofloxacin (21%) and cephalosporin (19%) accounted for the majority of prior antimicrobial therapy in TTSS- patients. There were no discernable differences in the prescription pattern of antibiotics between the two groups.

The average time to first antibiotic dose from a reported positive blood culture was 4.3 ± 1.2 hours. Empiric monotherapy for Gram negative organisms was prescribed for 17 (46%) and 28 (58%) of the TTSS+ and the TTSS- groups, respectively; (p=0.36). Piperacillin/tazobactam was the predominant agent used in both groups (n=49), followed by ciprofloxacin (n=19) and cefipime (n=13). Combination therapy consisted mainly of piperacillin/tazobactam plus ciprofloxacin (n=12), and imipenem plus gentamycin (n=6). Overall, fifteen (18%) out of 85 patients received inadequate antimicrobial therapy. Two patients in the TTSS+ and four patients in the TTSS- received no initial Gram-negative antibiotics directed against *P. aeruginosa* blood stream infection. The remaining nine were treated with antibiotics having recognized activity against *P. aeruginosa* but for which the bacteria were actually resistant by in vitro susceptibility testing.

The antimicrobial resistance profile of *P. aeruginosa* isolates are shown in Table 5. Fifty seven percent of isolates with TTSS+ phenotypes were resistant to two or more antimicrobial agents compared to 15% of TTSS- phenotype (p<0.001). A higher frequency of resistance in the TTSS+ isolates was observed to ciprofloxacin (59%), cefipime (35%), and gentamycin (38%) compared to TTSS- isolates. Piperacillin/tazobactam had the least antimicrobial resistance in both groups. ExoU secretory phenotype was present in 11 out of 22 (50%) of ciprofloxacin-resistant isolates and in 9 out of 14 (64%) gentamicin-resistant isolates. In comparison, ExoS was identified in 5 (23%) of ciprofloxacin-resistant isolates and in one of 14 (7%) gentamicin-resistant isolates.

Hospital course and outcome

During the course of hospitalization, 6 patients had recurrent *P. aeruginosa* bacteremia (Table 6). The average time between the primary and secondary blood stream infection isolates was 31 ± 10 days. All recurrent *P. aeruginosa* isolates were considered reinfection due to genetically distinct profile from the initial bacteremic isolates. There was no correlation between recurrence of bacteremia and secretory phenotypes.

The overall 30-day crude mortality was 32%. The 30-day and in-hospital mortality rates among patients with *P. aeruginosa* isolates that expressed TTSS+ protein were 46% and 57% compared with 21% and 25% among patients whose *P. aeruginosa* did not express any of the exotoxins ($p=0.03$ and 0.006 ; respectively). The survival curve also shows significant differences in the first 30-day cumulative probability of death after bacteremia between secretors and nonsecretors ($p=0.02$) (Figure 3). All TTSS+ patients who did not survive 30 days exhibited ExoU and/or ExoT phenotypes while none of the TTSS+ patients who survived the first 30 days had a *P. aeruginosa* isolate with ExoU phenotype. There was a trend toward higher ventilator-free days in the TTSS– group compared to the TTSS+ group (22.7 ± 17.9 versus 17.3 ± 13.6 days) but the difference did not reach statistical significance ($p=0.09$). Further, inadequate antimicrobial therapy was associated with worse 30-day outcome than those who had appropriate therapy irrespective of the TTSS phenotype (23% versus 4%, respectively; $p=0.03$). Cox regression analysis identified APACHE II, the presence of TTSS phenotypes and inadequate antimicrobial therapy as independent risk factors for 30-day mortality (Hazard ratio 2.6 (95% confidence interval [CI] 1.1–6.2, 2.9 (95% confidence interval [CI] 1.3–6.7) and 3.1 (95% CI 1.2–7.5), respectively) (Table 7).

DISCUSSION

The results of our study demonstrated that: 1) the prevalence of TTSS+ phenotype in *P. aeruginosa* bacteremia is common, with ExoU/ExoT representing the predominant phenotype; 2) there is a high association between antimicrobial resistance and TTSS+ phenotypes; and 3) type III protein secretion is associated with increased 30-day mortality in *P. aeruginosa* bacteremia.

Our investigation showed a prevalence of 44% of TTSS+ isolates in our study population based on the excretion of ExoU, ExoT, and ExoS. We have not investigated the presence of PcrV, PopB, or PopD because previous reports documented a lack of cytotoxicity of these proteins per se when tested in vitro (21, 22). The proportion of TTSS+ strains that exhibited exoU (48%) was higher than that reported by Berthelot et al (21) (28%) but close to that observed by Wareham et al. (23) (40%). In contrast, the secretion of ExoS (27%) was lower than the reported rate of 52% in the study by Barthelot et al. (21) This wide range of prevalence among bacteremic *P. aeruginosa* isolates with the type III secretory proteins may be difficult to elucidate in the absence of a surveillance database. However, the almost ubiquitous presence of *exoU*⁺ and *exoS*⁺ genes in *P. aeruginosa* blood stream isolates (24) suggests that the system is modulated by environmental cues that trigger the expression of TTSS genes. Unfortunately, further advance in this field is limited by the fact that TTSS expression is induced ex-vivo through manipulation of the external milieu and not assessed in vivo. The use of *in vivo* gene expression technologies (25) may hold promise in accurately determining the spatial and temporal control of these impressively complex regulatory cascades.

Previous studies have linked multidrug resistance in *P. aeruginosa* to TTSS phenotypes in ocular, blood, urine, and wound isolates (26, 27). Wong-Beringer and colleagues (27) studied this association by concentrating on fluoroquinolone-resistant clinical isolates of *P. aeruginosa*. Compared with *exoS*⁺ isolates, *exoU*⁺ isolates were more likely to be fluoroquinolone-resistant and to exhibit both a *gyrA* mutation and the efflux pump over-expressed phenotype. More importantly, almost all *exoU*⁺ strains secreted ExoU. Although we have not performed a genotypic analysis, our results are in agreement with these observations which suggest a co-selection of fluoroquinolone resistance and enhanced TTSS virulence in isolates harboring *exoU*⁺. We noted also a high frequency of resistance for gentamicin among isolates secreting ExoU but not for ExoS. This resistance appears to be

drug-specific rather than class-specific as the susceptibility to amikacin was not altered by the presence of the TTSS phenotypes.

Other investigators have demonstrated that the source of bacteremia, inadequate antimicrobial therapy, and severity of illness at the time of bacteremia are associated with a poor prognosis in *P. aeruginosa* infection (7, 28–30). Our study suggests that type III secretory phenotype should be added to the growing list of factors responsible for increased mortality from *P. aeruginosa* bloodstream infection. The presence of a functional TTSS has already been linked to bacterial persistence in the lungs, higher relapse rates, and increased mortality in patients with acute respiratory infections (13, 16, 31). Furthermore, secretion of type III proteins was associated with increased mortality in patients with a high bacterial burden in respiratory secretions but who failed to meet clinical criteria for the diagnosis of ventilator associated pneumonia (14). This is further substantiated in our study by the fact that despite adequate antimicrobial therapy for both TTSS+ and TTSS– groups initially, patient with TTSS+ phenotype had a worse prognosis, most notably during the first few days. The same observation was reported in a retrospective analysis of 115 *P. aeruginosa* bloodstream infections (7). The adequacy of the initial therapy did not influence survival up to receipt of the antibiogram. However, after censoring those patients that died during the first days, the risk of death for inadequate empirical treatment was significantly higher from the date of receipt of the antibiogram to day 30. Chamot et al. (7) did not examine the phenotype of these isolates but altogether these results suggest that a subpopulation of patients will die from *P. aeruginosa* bloodstream infection independently of their treatment. However, adequacy of empirical antimicrobial treatment influences the outcome of those patients that survive the first days following *P. aeruginosa* bloodstream infection.

There are several limitations to the present study that should be noted. First, we acknowledge the limitation of the retrospective design of the study which might introduce bias that we are unable to adjust for using the collected indices. Despite the presence of protocol-driven ICU management, changes in disease patterns and treatment strategies over the study period may have generated a time bias in our analysis favoring late participants. It is increasingly recognized that outcomes also depend on a variety of clinical and nonclinical factors that are not measured and therefore cannot be adjusted for in the available models. Second, the *P. aeruginosa* blood culture data were collected from a single site; institutional differences in prescribing patterns, antibiotic formularies, and patient populations may affect the applicability of these results to other institutions. Third, we cannot exclude the possibility that a number of strains classified as TTSS– phenotype may exhibit type III secretory phenotypes based on the release of PcrV. These strains are however less virulent *in vitro* than those responsible for excretion of ExoU, ExoS, or ExoT (21). Fourth, we have not assessed the expression of other virulence factors that may be co-expressed with TTSS phenotype.

In conclusion, expression of the type III phenotype confers an increased virulence in *P. aeruginosa* bacteremia. The association between ExoU phenotype and fluoroquinolone resistance needs to entail a prescription pattern that provides adequate coverage for suspected *P. aeruginosa* bacteremia in accordance with the local susceptibility profile. Interference with TTS function by chemical or biological inhibition and vaccination has great potential to reduce the morbidity and mortality associated with infection with these pathogens.

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Figure 1. Immunoblot analysis showing the type III protein secretion phenotypes of representative *P. aeruginosa* isolates. Individual isolates were grown under conditions that induced type III protein secretion. Immunoblot analysis was then performed on culture supernatants using a mixture of antisera against type III exotoxins.

Figure 2.

DNA genotyping of representative *Pseudomonas aeruginosa* isolates by repetitive-element-based polymerase chain reaction assay. Samples with identical banding patterns were considered to be related and assigned a single RAPD type. Samples with patterns that differed by one band were considered possibly related. Samples that differed by two or more bands were considered unrelated.

Figure 3.

Kaplan-Meier probability estimate for 30-day mortality of *P. aeruginosa* bacteremia according to type III secretion phenotype. The broken line represents patients with bacteremia caused by *P. aeruginosa* type III secretion phenotype; the solid line represents patients with bacteremia caused by type III non secretory phenotype.

Table 1

Baseline characteristics of the study population

	TTSS (+) (n=37)	TTSS (-) (n=48)	P value
Age, years	72.2±13.3	70.8±9.1	0.58
Gender (M/F)	34/3	47/1	0.31
Underlying disease, n(%)			
Diabetes mellitus	16 (43)	18 (38)	0.76
Malignancy	7 (19)	11 (23)	0.85
End-stage renal disease	8 (21)	14 (29)	0.59
Hemiplegia	4 (11)	1 (2)	0.16
Immunosuppression	9 (24)	6 (13)	0.28
Charlson Index	4.4±2.3	3.8±1.5	0.48
Source of infection, n(%)			
Respiratory	11 (29)	7 (17)	0.15
Urinary	3 (8)	1 (2)	0.31
Abdominal	5 (14)	9 (19)	0.72
Soft-tissue	1 (3)	5 (11)	0.23
Catheter	4 (11)	11 (23)	0.24
Unknown	13 (35)	15 (29)	0.89

Table 2

Clinical characteristics of the study population

	TTSS (+) (n=37)	TTSS (-) (n=48)	P value
Vital signs			
Systolic BP, <i>mmHg</i>	106±23	113±30	0.16
Temperature, °F	100.5±2.3	99.9±2.1	0.37
Baseline laboratory values			
White blood cells, <i>10⁹/L</i>	13.8±7.3	12.7±7.6	0.57
Blood glucose, <i>mg/dl</i>	180±119	195±135	0.48
Creatinine, <i>mg/dl</i>	1.7±1.1	1.6±0.9	0.79
Lactate, <i>mmol/L</i>	3.7±3.1	3.4±2.6	0.63
Arterial pH	7.32±0.11	7.35±0.09	0.28
APACHE II score	24.5±5.6	23.3±4.8	0.23
Therapeutic intervention			
Mechanical ventilation, <i>n (%)</i>	17 (46)	19 (39)	0.71
Need for vasopressors, <i>n (%)</i>	16 (43)	12 (25)	0.12
Norepinephrine dose in the first 48 hours, <i>µg/kg/min</i>	0.28±0.14	0.19±0.11	0.02
Hydrocortisone, <i>n (%)</i>	14 (38)	9 (19)	0.09
Vasopressin, <i>n (%)</i>	14 (37)	11 (23)	0.21
Adequate antibiotics, <i>n (%)</i>	34 (92%)	44 (91%)	0.73

Table 3

Distribution of type III secretion phenotypes according to suspected sites of infection

Phenotype	Respiratory	Abdominal	Urinary	Soft tissue	Catheter
ExoT	3	0	1	0	0
ExoS	1	1	0	0	1
ExoU/ExoT	6	1	1	1	3
ExoS/ExoT	1	3	1	0	0

Table 4

RAPD analysis of *P. aeruginosa* isolates

			Secretion Phenotype				
			None	ExoS+	ExoT+	ExoU+ ExoT+	ExoT+ ExoS+
Profile Type	P1	Unique	24	1	7	13	6
		P1	1	0	0	0	0
		P1a	1	0	0	0	0
	P2	P2	0	0	0	1	1
		P2a	0	0	1	0	0
		P2b	1	0	0	0	0
	P3		2	0	0	0	0
			0	0	0	3	0
	P5	P5	1	0	1	0	0
		P5a	1	0	0	0	0
	P6		2	0	0	0	0
			2	0	0	0	0
	P7	P7	1	0	0	0	0
		P7a	2	0	0	0	0
Profile Type	P8		1	1	0	0	0
			1	0	0	0	0
	P10	P10	1	0	0	0	1
		P10a	1	0	0	0	0
	P11	P11	1	0	0	0	0
		P11a	1	0	0	0	0
	P12		2	0	0	0	0
			1	0	0	0	0
	P13	P13	1	0	0	0	0
		P13a	1	0	0	0	0
	P14	P14	1	0	0	0	0
		P14a	0	0	0	1	0

[†] Lowercase letter indicates isolate differs from profile type by 1 band (possibly related)

Table 5Antimicrobial resistance pattern of *P. aeruginosa* isolates

Antibiotics, n (%)	TTSS (+) (n=37)	TTSS (-) (n=48)	P value
Piperacillin/tazobactam	2 (5)	0	0.19
Cefipime	13 (35)	7 (15)	0.05
Imipenem-cilastatin	3 (8)	6 (13)	0.72
Ciprofloxacin	22 (59)	2 (4)	<0.001
Gentamycin	14 (38)	6 (13)	0.01
Amikacin	2 (5)	3 (6)	1.0

Table 6

Recurrent *P. aeruginosa* bacteremia

Patient	Primary isolate			Time to second positive blood culture (days)	Secondary isolate	
	RAPD Profile	Secretion Phenotype	ExoT/ExoS		RAPD Profile	Secretion Phenotype
1	U		ExoT/ExoS	26	U	none
2	U		none	42	P7	none
3	P11		none	44	P8	none
4	P3		none	23	P5	none
5	U		none	29	U	none
6	U		none	19	P1	none

U= Unique profile

Table 7

Cox regression analysis of 30-day mortality

Covariate	b	SE	P value	HR	95% CI of HR
APACHE II	0.9683	0.4432	0.0289	2.6336	1.1097 to 6.2498
Prior antibiotics	0.6561	1.0340	0.5258	1.9272	0.2566 to 14.4754
TTSS+	1.0669	0.4254	0.0121	2.9064	1.2680 to 6.6618
Inadequate therapy	1.1174	0.4606	0.0153	3.0568	1.2450 to 7.5054

SE=Standard error

HR= Hazard ratio

CI = Confidence interval

b = coefficient of covariates