MAJOR ARTICLE

Influence of Virulence Genotype and Resistance Profile in the Mortality of *Pseudomonas aeruginosa* Bloodstream Infections

Carmen Peña,[1] Gabriel Cabot,[2] Silvia Gómez-Zorrilla,[1] Laura Zamorano,[2] Alain Ocampo-Sosa,[3] Javier Murillas,[2] Benito Almirante,[4] Virginia Pomar,[5] Manuela Aguilar,[6] Ana Granados,[7] Esther Calbo,[8] Jesús Rodríguez-Baño,[9] Fernando Rodríguez-López,[10] Fe Tubau,[1] Luis Martínez-Martínez,[3,11] and Antonio Oliver[2]; for the Spanish Network for Research in Infectious Diseases (REIPI)

[1]Servicio de Enfermedades Infecciosas, Hospital Universitario de Bellvitge-IDIBELL, Barcelona, [2]Unidad de Investigación, Servicio de Microbiología y Servicio de Medicina Interna, Hospital Universitario de Son Espases, Instituto de Investigación Sanitaria de Palma, Palma de Mallorca, [3]Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla-IFIMAV, Santander, [4]Servicio de Enfermedades Infecciosas, Hospital Universitario Vall d'Hebrón, [5]Unidad de Enfermedades Infecciosas, Hospital Santa Creu i Sant Pau, Barcelona, [6]Servicio de Enfermedades Infecciosas, Hospital Universitario Virgen del Rocío, Sevilla, [7]Sección de Enfermedades Infecciosas, Consorci Hospitalari Parc Taulí, Sabadell, [8]Sección de Enfermedades Infecciosas, Hospital Mutua de Terrasa, [9]Sección de Enfermedades Infecciosas, Hospital Universitario Virgen Macarena, Sevilla, [10]Servicio de Microbiología Infecciosas, Hospital Universitario Reina Sofía-IMIBIC, Córdoba, and [11]Departamento de Biología Molecular, Universidad de Cantabria, Santander, Spain

Background. The type III secretion system (TTSS) is a major virulence determinant of *Pseudomonas aeruginosa*. The objective of this study was to determine whether the TTSS genotype is a useful prognostic marker of *P. aeruginosa* bacteremia mortality. We also studied the potential association between TTSS genotypes and multidrug-resistant (MDR) profiles, and how this interaction impacts the outcome of bloodstream infections.

Methods. We performed a post hoc analysis of a published prospective multicenter cohort of *P. aeruginosa* bloodstream infections. The impact in mortality of TTSS genotypes (*exoS*, *exoT*, *exoU*, *and exoY* genes) and resistance profiles was investigated. Cox regression analysis was used to control for confounding variables.

Results. Among 590 patients, the 30-day mortality rate was 30% (175 patients), and 53% of them died in the first 5 days (early mortality). The unadjusted probabilities of survival until 5 days was 31.4% (95% confidence interval [CI], 17.4%–49.4%) for the patients with *exoU*-positive isolates and 53.2% (95% CI, 44.6%–61.5%) for *exoU*-negative isolates (log rank $P = .005$). After adjustment for confounders, *exoU* genotype (adjusted hazard ratio [aHR], 1.90 [95% CI, 1.15–3.14]; $P = .01$) showed association with early mortality. In contrast, late (30-day) mortality was not influenced by TTSS genotype but was independently associated with MDR profiles (aHR,1.40 [95% CI, 1.01–1.94]; $P = .04$). Moreover, the *exoU* genotype (21% of all isolates) was significantly less frequent (13%) among MDR strains (particularly among extensively drug-resistant isolates, 5%), but was positively linked to moderately resistant (1–2 antipseudomonals) phenotypes (34%).

Conclusions. Our results indicate that the *exoU* genotype, which is associated with specific susceptibility profiles, is a relevant independent marker of early mortality in *P. aeruginosa* bacteremia.

Keywords. type III secretion system; multidrug resistance; virulence; bloodstream infections; *Pseudomonas aeruginosa*.

Received 10 July 2014; accepted 24 October 2014; electronically published 6 November 2014.

Correspondence: Antonio Oliver, PhD, Servicio de Microbiología, Hospital Son Espases, Ctra Valldemossa 79, 07010 Palma de Mallorca, Spain (antonio.oliver@ssib.es).

Clinical Infectious Diseases® 2015;60(4):539–48

DOI: 10.1093/cid/ciu866

Pseudomonas aeruginosa (PA) is a common cause of nosocomial infections, which are often severe. Despite improvements in hospital care, PA bacteremia remains fatal in about 30% of cases [1, 2]. Poor outcomes in PA infections have been associated with factors related to the host, the antibiotic treatment, and the microorganism.

Septic shock, initial site of infection, and immunosuppression at the time of PA bacteremia are among the host factors implicated in increased mortality [3, 4]. In addition, the presence of multidrug-resistant (MDR) strains reduces the treatment options and enhances the risk of inadequate empirical therapy, although the excess of mortality explained by antimicrobial resistance is not evident during the first days after onset of bacteremia [1]. These findings might be related to the alteration of fitness caused by resistance genes or mutations, potentially reducing the virulence of the infecting microorganism [5].

Moreover, a large number of deaths occurred within the first 24–72 hours after the diagnosis of PA infection despite the use of adequate antimicrobial therapy [6, 7], and although the reasons for the PA bacteremia mortality are multifactorial, the intrinsic virulence of PA likely plays a major role. One of the most relevant virulence determinants of PA is the type III secretion system (TTSS) [8]. This secretion system injects potent cytotoxins, including ExoS, ExoT, ExoU, or ExoY, into eukaryotic cells [9]. The production of each of the different enzymes determines a distinct host tissue injury, with ExoU being the one determining a greater impact in bacterial virulence [8]. Moreover, the distribution of the genes encoding these cytotoxins is not uniform among *P. aeruginosa* strains, and some of them, particularly *exoS* and *exoU*, appear to be mutually exclusive. Therefore, defined clonal lineages, including the widespread MDR high-risk clones [10], are expected to be linked to specific TTSS genotypes, and this linkage may play a major role in their intrinsic virulence levels [11]. Moreover, recent studies suggest the existence of an association between defined TTSS genotypes and certain antibiotic resistance profiles [12–14].

Although several studies have investigated the presence of TTSS genes in PA strains involved in human diseases [13, 15], to our knowledge, only a limited number of studies with small sample sizes have evaluated the impact of these virulence mechanisms in the outcome of PA respiratory or bloodstream infections [6, 14, 16–18].

Therefore, we designed an analysis of a large multicenter, prospective cohort of PA bacteremia [1], with the main objective of investigating the impact of the TTSS genotype on PA bacteremia mortality. In addition, we studied the potential association between TTSS genotypes and MDR profiles, and how this interaction may impact the outcome of PA bloodstream infections.

MATERIALS AND METHODS

Setting and Design

We performed a post hoc analysis of a cohort of patients with PA bacteremia from a large multicenter study in Spain [1]. Clinical, microbiological, and outcome data were used as previously reported [1].Only the first bacteremia episode for each patient was included in the analysis. The study was approved by the local ethics committees of the participating centers.

Variables and Definitions

The following data were recorded: age and sex; comorbidities and severity of underlying diseases calculated using the Charlson comorbidity index [19] and severity of illness estimated by the Simplified Acute Physiology Score (SAPS II) in intensive care unit patients [20]; the presence of neutropenia (absolute granulocyte count of <500/mL) and the use of immunosuppressive therapy (chemotherapy, radiotherapy and/or immunosuppressive drugs during the bacteremia presentation); source of bacteremia; severity of acute illness at presentation according to the Pitt bacteremia score [21]; presence of septic shock and multiorgan dysfunction syndrome at presentation and at 48 hours [22]; and antimicrobial treatment received. The source of bacteremia was divided into 2 categories: low risk (urinary tract, vascular catheter, and pancreaticobiliar) and high risk (all other sources) [23]. Antimicrobial therapy was considered adequate when the PA isolate was susceptible to the antimicrobial prescribed and the dose was in accordance with current medical standards. For outcomes, late mortality was defined as death from any cause occurring in the 30 days after the onset of PA bacteremia; mortality was considered as early mortality for patients who died within the first 5 days.

Microbiological Studies

Assessment of the Antimicrobial Susceptibility Profile

Detailed information describing the microbiological studies has been reported previously [2]. The following antimicrobial agents were tested: aztreonam, ceftazidime, cefepime, piperacillin, piperacillin-tazobactam, imipenem, meropenem, gentamicin, tobramycin, amikacin, levofloxacin, ciprofloxacin, and colistin. Clinical categories were determined according to the breakpoints defined by the Clinical and Laboratory Standards Institute [24].

Phenotype stratification of PA isolates was made in accordance with recent standard definitions [25]. MDR PA was defined as a strain nonsusceptible to ≥1 agent in ≥3 antipseudomonal antimicrobial categories. Extensively drug-resistant (XDR) PA was defined as nonsusceptible to ≥1 agent in all but ≤2 antipseudomonal antimicrobial categories; thus, a XDR isolate was also included in MDR category [25]. To study the specific epidemiology of XDR isolates, MDR isolates were divided into XDR and MDR non-XDR. Finally, PA isolates nonsusceptible to ≥1 agent in 1 or 2 antimicrobial categories were considered moderately resistant. Thus, 4 phenotypes of PA isolates were considered: susceptible, moderately resistant, MDR non-XDR, and XDR.

TTSS Polymerase Chain Reaction Genotyping

Polymerase chain reaction (PCR) assays for detection of *exoS*, *exoT*, *exoU*, and *exoY* genes were performed with primers and

Table 1. Baseline Characteristics of Patients With *Pseudomonas aeruginosa* Bacteremia According to Type III Secretion System Genotype

Characteristic	*exoU* (n = 126)	*exoS* (n = 443)	*P* Value[a]	*exoU*$^{-}$/*exoS*$^{-}$ (n = 21)	*P* Value[b]
Age, y, mean ± SD	65.0 ± 16.90	65.3 ± 16.35	.88	59.6 ± 21.84	.19
Male sex	86 (68)	301 (68)	.95	15 (71)	.77
Acquisition					
Nosocomial	74 (59)	254 (57)	.39	16 (76)	.06
Healthcare related	48 (38)	167 (37)	.46	3 (14)	.01
Community	4 (3)	22 (5)	.07	2 (10)	.12
PA phenotype					
Non-MDR	104 (82.5)	297 (67%)	.001	21 (100%)	.03
Susceptible	56 (44)	209 (47)	.58	15 (71)	.02
Moderately resistant	48 (38)	88 (20)	<.001	6 (29)	.40
MDR	22 (17.5)	146 (33)	.001	0	. . .
Non-XDR	18 (14)	69 (16)	.72	0	. . .
XDR	4 (3)	77 (17)	<.001	0	. . .
ICU stay	30 (24)	121 (27)	.43	6 (29)	.64
SAPS score, mean ± SD	47.3 ± 19.4	42.3 ± 17.5	.17	44.5 ± 15.0	.74
Charlson index, median (IQR)	3 (1–4)	2 (2–5)	.80	2 (1–3)	.09
Underlying condition					
Diabetes	41 (32.5)	110 (25)	.08	5 (24)	.42
Chronic lung disease	16 (13)	68 (15)	.46	2 (9.5)	1.00
Heart disease	26 (21)	77 (17)	.40	4 (19)	1.00
Solid malignancy	37 (29)	126 (28)	.84	6 (29)	.94
Hematologic malignancy	14 (11)	74 (17)	.12	2 (9.5)	1.00
Chronic renal failure	25 (20)	66 (15)	.18	3 (14)	.76
Chronic neurologic disease	10 (8)	31 (7)	.72	0	. . .
Cirrhosis	6 (5)	21 (5)	.99	2 (9.5)	.32
Immunosuppression	30 (24)	118 (27)	.52	4 (19)	.78
Neutropenia	5 (4)	25 (6)	.46	2 (9.5)	.26
Origin of bacteremia					
High-risk bacteremia	57 (45)	217 (49)	.46	9 (43)	.84
Unknown	31 (25)	123 (28)	.49	7 (33)	.43
Respiratory tract	9 (7)	54 (12)	.11	2 (9.5)	.66
Abdominal	10 (8)	15 (3)	.02	0	. . .
Soft tissue	4 (3)	16 (4)	.82	0	. . .
Other(s)	3 (2)	9 (2)	.81	0	. . .
Low-risk bacteremia	69 (55)	226 (51)	.46	12 (57)	.84
Vascular catheter	26 (21)	82 (18.5)	.58	6 (29)	.45
Urinary tract	39 (31)	116 (26)	.27	5 (24)	.47
Pancreaticobiliary	4 (3)	28 (6)	.27	1 (5)	.55
Clinical presentation					
Pitt score, median (IQR)	1 (0–3)	1 (0–3)	.47	1 (0–3)	.78
Shock initial	26 (21)	83 (19)	.63	5 (24)	.74
MODS initial	14 (11)	45 (10)	.75	3 (14)	.71
Shock/MODS at 48 h	4 (3)	27 (6)	.27	0	. . .
Outcome					
Late mortality (30-day)	34 (27)	133 (30.5)	.47	8 (38)	.31
Early mortality (5-day)	25 (73.5)	62 (47)	.005	6 (75)	.93

Data are presented as No. (%) unless otherwise specified.

Abbreviations: ICU, intensive care unit; IQR, interquartile range; MDR, multidrug resistant; MODS, multiorgan dysfunction syndrome; PA, *Pseudomonas aeruginosa*, SAPS, Simplified Acute Physiology Score; SD, standard deviation; XDR, extensively drug resistant.

[a] *exoU* vs *exoS*.

[b] *exoU* vs *exoU*$^{-}$/*exoS*$^{-}$.

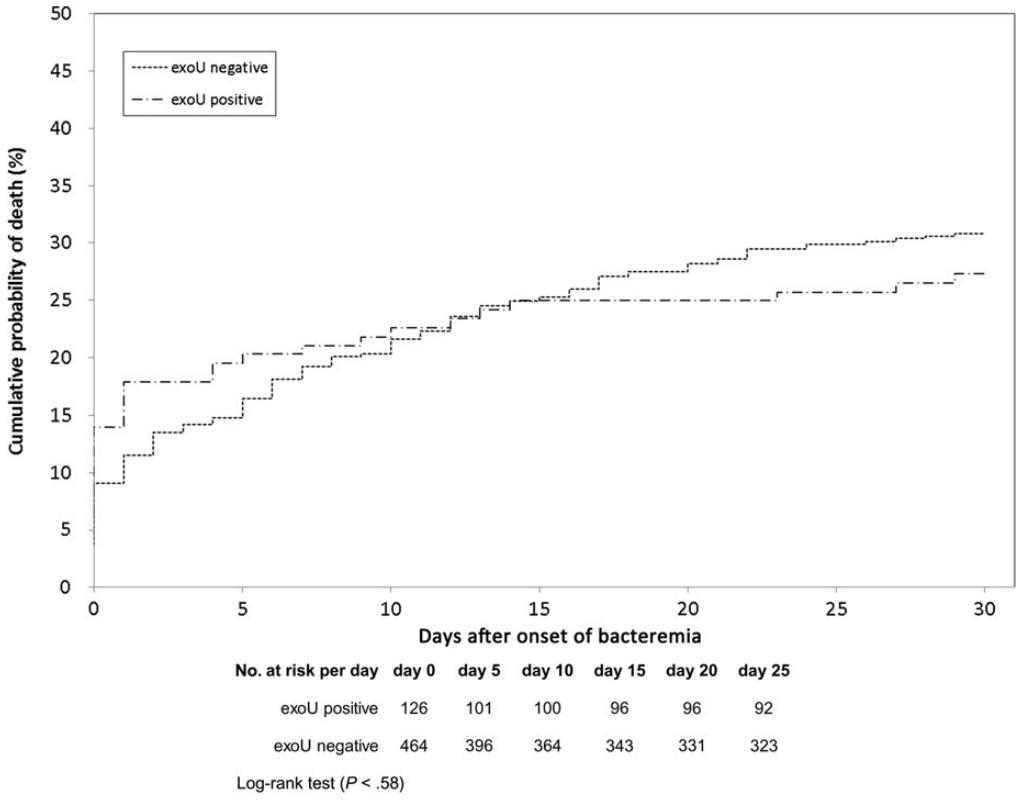

Figure 1. Probability estimate for 30-day mortality of *Pseudomonas aeruginosa* bacteremia according to type III secretion system *exoU* genotype.

protocol described by Feltman et al [15] with slight modifications. PCR reactions were performed with AmpliTaq DNA polymerase (Applied Biosystems) in a DNA thermal cycler (Arktic Thermal Cycler, Thermo Fisher), under the following conditions: denaturation for 5 minutes at 94°C, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, and a final extension step of 10 minutes at 72°C.

Molecular Typing

Partial data on clonal relatedness were available from previous studies [2, 26, 27]. For the present work, all XDR isolates were analyzed through multilocus sequence typing (MLST) to identify prevalent high-risk clones [28, 29].

Statistical Analysis

Continuous variables were analyzed using a 2-tailed Student *t* test for normally distributed variables and the Mann–Whitney test for nonnormally distributed variables. Means and standard deviations are presented for normal data and medians with interquartile ranges for nonnormally distributed data. Survival curves were constructed by means of the Kaplan–Meier method and log-rank test to estimate the risk of death according to TTSS genotype and according to susceptibility profile. The outcome evaluated was death, and the date of the initial PA isolate was considered as time 0. Patients were monitored until day 30 after bacteremia to determine early (5-day) and late (30-day) mortality; cases were censored from analysis either because of death or transfer to another hospital (those lost to follow-up were censored on the last follow-up day on which they were known to be alive).

To control for confounding effects of TTSS genotype (exposure) on time to mortality (early mortality and late mortality), we used multivariate adjusted hazard ratios (aHRs) with Cox regression. To assess effect modification, possible interaction terms between the exposure variable (TTSS genotype) and covariates were examined and maintained in the model depending on the results of the significance test ($P < .05$). In the crude analysis, variables that were associated with exposure with a P value <.20 were candidates for multivariate analysis. Statistical tests were 2-tailed; $P \leq .05$ was considered significant. All statistical analyses were performed with the SPSS package version 15.0.

RESULTS

Epidemiological and Clinical Characteristics According to TTSS Genotype

The TTSS genotype was characterized for 593 patients with a single episode of PA bacteremia. All strains were positive for

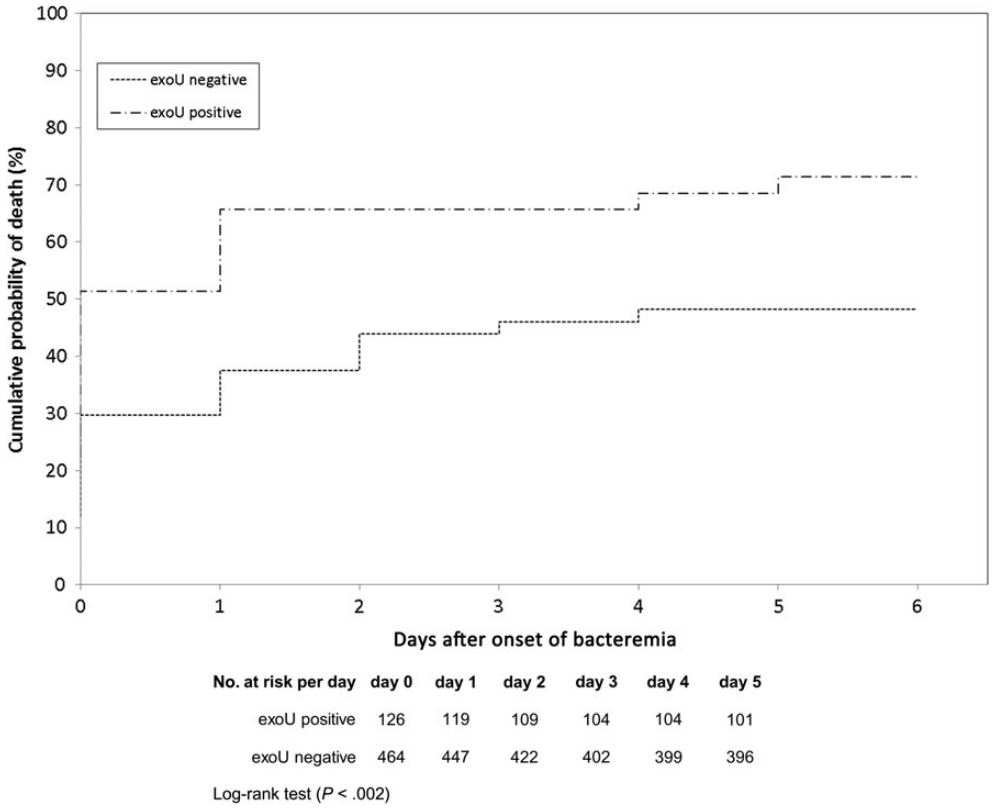

Figure 2. Probability estimate for 5-day mortality of *Pseudomonas aeruginosa* bacteremia according to type III secretion system *exoU* genotype.

either *exoU* (126 strains [21%]) or *exoS* (443 strains [75%]), except for 3 (0.5%) and 21 (3.5%) strains that were positive or negative for both genes, respectively. Concomitantly, the presence of *exoT* and *exoY* was documented in nearly all PA strains (591 [98%] and 566 [95%], respectively).

The clinical and epidemiological characteristics according to TTSS genotype are shown in Table 1. Because *exoT* and *exoY* genotypes were nearly universally positive, these genes were not included in the analysis. Moreover, the 3 bacteremia episodes that exhibited both the *exoU* and *exoS* genotypes were excluded from the analysis given the very limited number of cases. Therefore, 3 major groups (590 patients) were analyzed and included in Table 1: $exoU^+/exoS^-$ (n = 126), $exoU^-/exoS^+$ (n = 443), and $exoU^-/exoS^-$ (n = 21). There were no significant differences in demographic data, underlying comorbidities, or presentation of severity of acute illness with the exception of the abdominal source of PA bacteremia that was more frequent in $exoU^+/exoS^-$ episodes than among $exoU^-/exoS^+$ cases (8% vs 3%; $P = .02$).

Crude Impact of TTSS Genotype in Mortality

As shown in Table 1, the TTSS genotype determined no significant differences in late (30-day) mortality. On the other hand, early mortality among patients with $exoU^+/exoS^-$ isolates was 73.5% compared with 47% among patients infected by $exoU^-/exoS^+$ strains ($P = .005$).

The overall 30-day mortality rate was 30% (175 patients), and 53% (93 patients) of them died in the first 5 days of bacteremia onset. The survival curve showed no significant differences in the 30-day cumulative probability of death (Figure 1) between the PA bacteremia isolates that were positive or negative for *exoU*, with the unadjusted probabilities of survival until day 30 being 69.5% (95% confidence interval [CI], 60.6%–77.2%) for $exoU^+$ patients, and 69.1% (95% CI, 64.6%–73.3%) in the $exoU^-$ group (log-rank $P = .58$). Nevertheless, the cumulative survival until day 5 (Figure 2) was significantly different between the PA episodes that were positive or negative for *exoU*. The unadjusted probabilities of survival until day 5 were 31.4% (95% CI, 17.4%–49.4%) for the patients with $exoU^+$ PA isolates and 53.2% (95% CI, 44.6%–61.5%) in the PA bacteremia by $exoU^-$ strains (log-rank $P = .002$).

Interplay Between TTSS Genotype and Drug Resistance Profiles

MDR phenotype was documented in 168 (28%) of the PA blood isolates, of which 87 were MDR non-XDR (52% of MDR and 15% of all isolates), and 81 were XDR (48% of MDR and 14% of all isolates). Susceptible phenotype was documented in 280 (47%), and the remaining 142 (24%) were stratified as

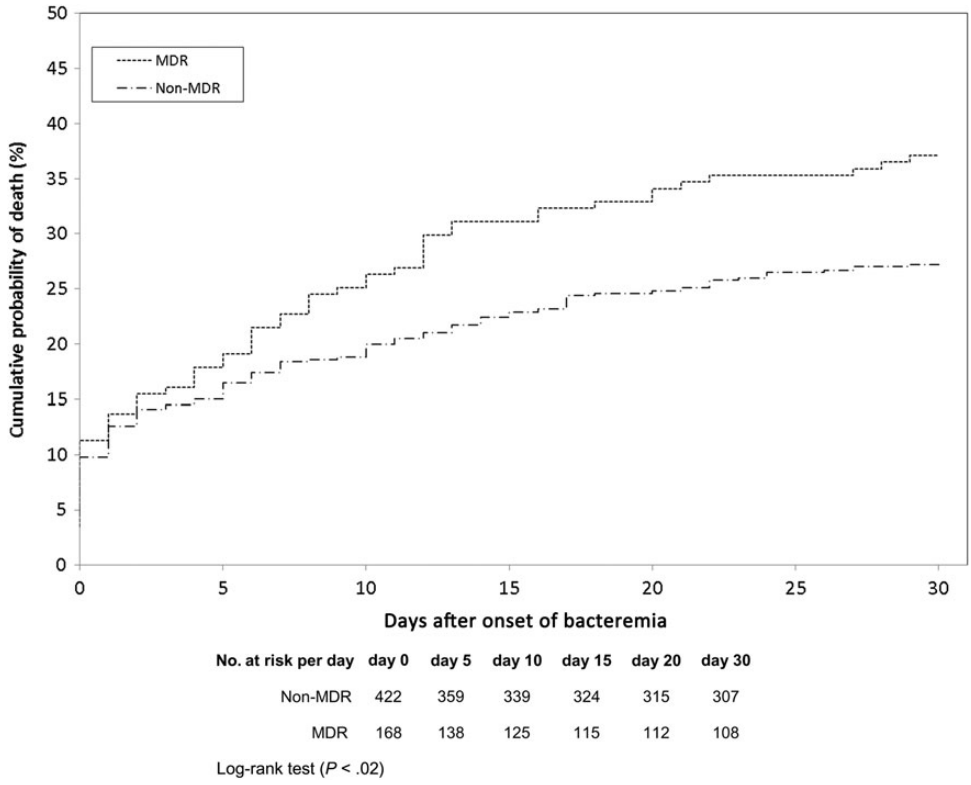

Figure 3. Probability estimate for 30-day mortality of *Pseudomonas aeruginosa* bacteremia according to resistance phenotype. Abbreviation: MDR, multidrug resistant.

moderately resistant strains. Among the 4 different phenotypes, the *exoS* genotype was detected in 209 (75%) of susceptible isolates, in 88 (62%) of moderately resistant isolates, in 69 (79%) of MDR non-XDR strains, and in 77 (95%) of XDR PA strains. In contrast, the *exoU* genotype was detected in 56 (20%) of susceptible strains, in 48 (34%) of moderately resistant strains, in 18 (21%) of MDR non-XDR strains, and in 4 (5%) of XDR isolates. The 3 $exoU^+/exoS^+$ isolates showed the susceptible phenotype.

As shown in Table 1, $exoS^+$ isolates were more frequently MDR than $exoU^+$ isolates (33% vs 17.5%; $P = .001$), and obviously the other way around for the non-MDR phenotype (67% vs 82.5%). However, within the non-MDR phenotype, a significant association was observed between *exoU* genotype and moderately resistant PA strains (38% vs 20%; $P < .001$) but not for fully susceptible strains (44% vs 47%). Likewise, within the MDR phenotype, $exoU^+$ isolates were significantly less frequent among XDR isolates (3% vs 17%; $P < .001$) but not among MDR non-XDR isolates (14% vs 16%).

In summary, the *exoU* genotype was positively linked to the moderately resistant phenotype and negatively linked to the XDR phenotype. According to our previous molecular typing data on selected isolates [26, 27], clonal diversity was extremely high (nearly each isolate representing a different clone) among the moderately resistant phenotype isolates and, therefore, the excess of $exoU^+$ strains is apparently not a consequence of the presence of specific clones. On the other hand, according to our previous data, clonal diversity was expected to be much more limited among XDR strains, since this phenotype is frequently linked to the so-called international high-risk clones. Thus, we analyzed by MLST all 81 XDR isolates and found, as expected, that the vast majority of the isolates belonged to previously identified high-risk clones, including sequence type (ST) 175 (n = 62), ST111 (n = 9), ST235 (n = 2), and ST244 (n = 2). Remarkably, of these high-risk clones, only ST235, represented by just 2 isolates, was positive for *exoU*, explaining the negative association between this TTSS gene and XDR phenotypes.

Crude Impact of MDR Phenotype in Mortality

Figure 3 shows the unadjusted probabilities of survival until day 30; survival was significantly (log-rank $P = .02$) higher for patients infected by non-MDR (72.9% [95% CI, 68.3%–77.1%]) than MDR strains (62.8% [95% CI, 55.0%–70.1%]). However, the probabilities of survival until day 5 (Figure 4) in non-MDR bacteremia (44.7% [95% CI, 35.5%–54.3%]) vs the MDR group (53.2% [95% CI, 40.2%–65.8%]) showed no significant differences (log-rank $P = .28$).

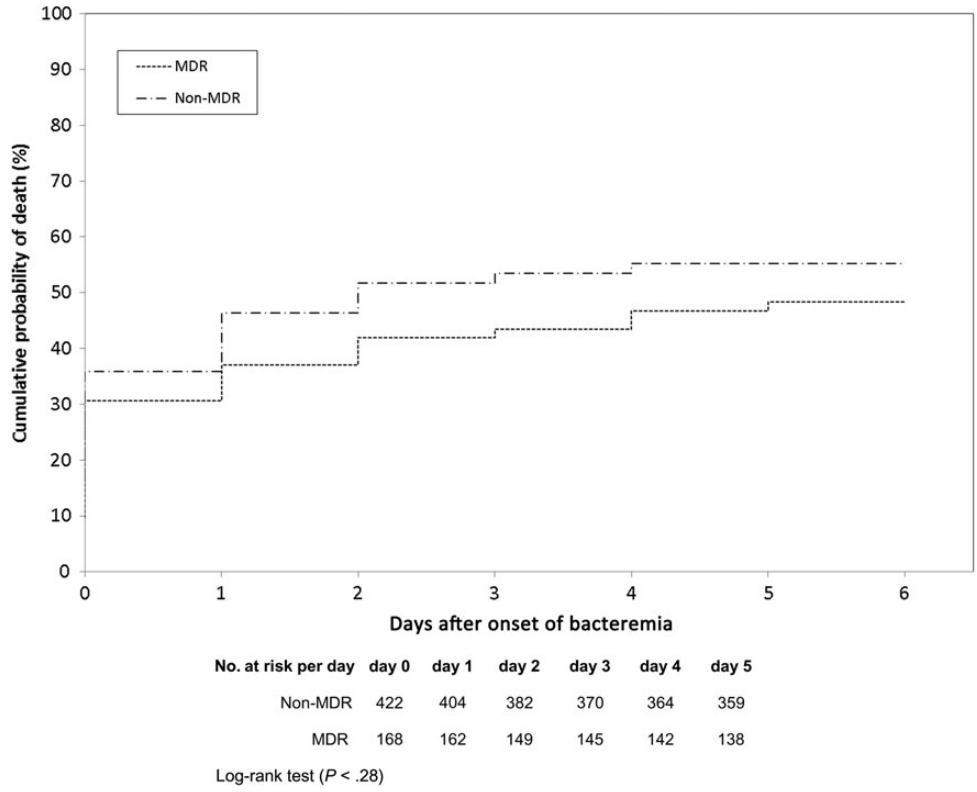

Figure 4. Probability estimate for 5-day mortality of *Pseudomonas aeruginosa* bacteremia according to resistance phenotype. Abbreviation: MDR, multidrug resistant.

Outcome

Early Outcome: 5-Day Mortality

Ninety-three patients died: 45 (48%) patients with PA bacteremia caused by susceptible strains, 18 (19.5%) for PA moderately resistant strains, 17 (18.5%) caused by MDR non-XDR, and the remaining 13 (14%) for XDR strains. Unadjusted and adjusted 5-day mortality for patients with PA bacteremia is shown in Table 2. After adjustment for significant variables, the early mortality was associated (aHR, 1.90 [95% CI, 1.15–3.14]; $P = .01$) with *exoU*$^+$ isolates (Table 2). Because a high clonal relatedness was observed among MDR/XDR isolates, we performed also the analysis of the impact of the *exoU* genotype on early mortality in the non-MDR group, which is mostly nonclonal as described above. Similar to the complete cohort, non-MDR *exoU*$^+$ isolates were significantly associated with early mortality (aHR, 2.37 [95% CI, 1.32–4.27]; $P = .004$).

Late Outcome: 30-Day Mortality

A total of 175 patients died: 78 (45%) patients infected by PA-susceptible strains, 35 (20%) by moderately resistant strains, 32 (18%) by MDR non-XDR strains, and 30 (17%) by XDR isolates.

Crude analysis showed that *exoU* genotype had no impact on 30-day mortality. Multivariate analysis, adjusted by the variables associated with worse prognosis, revealed MDR as one of the predictors of late mortality (Table 3).

DISCUSSION

The interconnections between antimicrobial resistance and virulence traits and how they may impact the outcome of bacterial infections is a subject of growing interest [30, 31]. In this work we analyzed the interplay between the TTSS, MDR phenotypes, and outcome of bloodstream infections.

As expected, *exoT* and *exoY* genes were detected in the vast majority of strains, but the presence of *exoS* and *exoU* was nearly mutually exclusive. Overall, 75% of the strains were *exoS*$^+$ and 21% *exoU*$^+$. Our findings are therefore in agreement with previous studies showing that *exoU*$^+$, ranging from 10% to 40%, are less frequent than *exoS*$^+$ (60%–90%) among PA clinical strains [12, 13, 15, 32, 33].

A number of previous studies with small sample sizes have evaluated the impact of TTSS in the outcome of PA respiratory or bloodstream infection [6, 14, 16–18]. Most of them evaluated whether the involved PA strains secreted (as evidenced by immunoblotting) or not TTSS cytotoxins in vitro and concluded that strains that produced at least 1 of them, designated

Table 2. Cox Regression Analysis of Predictor Factors for Early Mortality of Patients With *Pseudomonas aeruginosa* Bloodstream Infection

	Crude Analysis		Adjusted Analysis	
Variables	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age				
≤65 y				
>65 y	0.95 (.62–1.45)	.81	1.16 (.72–1.88)	.53
Sex				
Female				
Male	1.28 (.82–2.00)	.27	1.35 (.83–2.20)	.22
Non-MDR *P. aeruginosa* susceptibility				
No				
Yes	1.23 (.79–1.90)	.34		
TTSS *exoU*				
No				
Yes	1.81 (1.24–3.16)	.004	1.90 (1.15–3.14)	.01
Underlying disease				
Charlson index ≤2				
Charlson index ≥3	1.77 (1.13–2.78)	.011	2.01 (1.21–3.34)	.007
Source of bacteremia				
Low risk				
High risk	2.51 (1.44–4.39)	.012	1.94 (1.06–3.52)	.03
Immunosuppression				
No				
Yes	1.03 (.68–1.56)	.89		
Pitt bacteremia score				
<2				
≥2	3.63 (1.97–6.67)	<.001	2.86 (1.44–5.69)	.003
Initial shock				
No				
Yes	1.96 (1.30–2.96)	.001	0.83 (.49–1.39)	.48
Initial MODS				
No				
Yes	1.79 (1.15–2.75)	.010	0.93 (.55–1.59)	.80
Shock/MODS 48 h				
No				
Yes	2.59 (1.62–4.15)	<.001	2.47 (1.42–4.29)	.001
Adequate empiric antibiotic				
No				
Yes	0.89 (.57–1.39)	.62		

Abbreviations: CI, confidence interval; HR, hazard ratio; MDR, multidrug resistant; MODS, multiorgan dysfunction syndrome; TTSS, type III secretion system.

Table 3. Cox Regression Analysis of Predictor Factors for 30-Day Mortality of Patients With *Pseudomonas aeruginosa* Bloodstream Infection

	Crude Analysis		Adjusted Analysis	
Variables	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age				
≤65 y				
>65 y	1.52 (1.12–2.08)	.008	1.94 (1.39–2.70)	<.001
Sex				
Female				
Male	1.04 (.75–1.42)	.81	0.89 (.64–1.24)	.51
MDR *P. aeruginosa* susceptibility				
No				
Yes	1.42 (1.04–1.93)	.026	1.40 (1.01–1.94)	.04
TTSS *exoU*				
No				
Yes	0.90 (.62–1.30)	.58		
Underlying disease				
Charlson index ≤2				
Charlson index ≥3	1.71 (1.26–2.32)	<.001	1.90 (1.37–2.64)	<.001
Source of bacteremia				
Low risk				
High risk	2.99 (2.17–4.12)	<.001	2.22 (1.56–3.16)	<.001
Immunosuppression				
No				
Yes	1.90 (1.40–2.58)	<.001	1.24 (.89–1.72)	.20
Pitt bacteremia score				
<2				
≥2	4.14 (2.99–5.75)	<.001	2.10 (1.46–3.04)	<.001
Shock/MODS 48 h				
No				
Yes	7.3 (5.36–9.96)	<.001	5.29 (3.67–7.61)	<.001
Adequate definitive antibiotic				
No				
Yes	1.33 (.71–2.49)	.44		

Abbreviations: CI, confidence interval; HR, hazard ratio; MDR, multidrug resistant; MODS, multiorgan dysfunction syndrome; TTSS, type III secretion system.

"secretors," were associated with a worse outcome. Additionally, recent data show that the *exoU* genotype was associated with higher probability of developing pneumonia in patients with respiratory cultures positive for PA [14].

Therefore, in this work we asked whether the TTSS genotype could be used as prognostic marker. For this purpose, we analyzed a large multicenter prospective cohort of PA bacteremia. Indeed, the multivariate analysis performed showed for the first time that the *exoU* genotype is independently associated with increased risk of early mortality of PA bloodstream infections. In contrast, late mortality was not influenced by TTSS genotype but was significantly higher among patients infected by MDR strains.

As expected, underlying disease severity, source of bacteremia, and severity of illness at the time of bacteremia are

associated with a poor prognosis, most notably during the first few days if the infection was due to PA strains with *exoU* genotype. Despite a strong association of *exoU* genotype and early mortality, no significant correlation was observed between the presence of this TTSS genotype and shock or development of organ failure. These findings may indicate that the *exoU* genotype is important but not essential in the development of severe clinical presentation, and that the complex interplay between subsequent inflammatory host response and/or underlying host condition may affect the severity of PA infection.

We also investigated the potential association between TTSS genotypes and resistance profiles. Interestingly, we found that the *exoU* genotype was significantly more frequent among non-MDR strains. However, a closer analysis revealed that the *exoU* genotype was positively linked to the moderately resistant phenotype and negatively linked to the XDR phenotype. Because clonal diversity was extremely high among the moderately resistant phenotype isolates, the excess of $exoU^+$ strains is apparently not consequence of the presence of specific clones. Overall, the *exoU* genotype was detected in 21% of susceptible isolates but up to 34% of moderately resistant isolates. Regarding individual antibiotics among moderately resistant isolates, the prevalence of *exoU* genotype was highest among carbapenem-resistant isolates (48% for imipenem) followed by fluoroquinolone-resistant (32% for ciprofloxacin) and cephalosporin-resistant (31% for ceftazidime) isolates; in contrast, the *exoU* genotype was particularly infrequent among aminoglycoside-resistant isolates (6% for gentamicin). Thus, our results support and expand recent findings suggesting and association between the TTSS and fluoroquinolone resistance [12–14, 34], and determines a major step forward for understanding the interplay between resistance and virulence.

In contrast, the negative association between the *exoU* genotype and XDR phenotypes was determined by the widespread international high-risk clones ST175 and ST111 [26, 27, 35, 36], which were all $exoU^-/exoS^+$. It should be noted, however, that there is a third international high-risk clone, the $exoU^+/exoS^-$ ST235, which, although very infrequent in our series, has caused numerous outbreaks worldwide and is associated with a particularly poor outcome [11, 35, 37–39]. Thus, a high prevalence of this high-risk clone could have determined a significantly higher impact of MDR/XDR strains in mortality. Therefore, the specific clonal epidemiology of XDR strains in our series might be considered a limitation for the universal application of the study, but taken together, these results indicate that for analyzing the impact of XDR strains on the outcome of infections, the specific clonal types and, in particular, their TTSS genotype should be considered. Likewise, according to recent data [40], correlation between serotypes, clonal lineages, and TTSS genotypes should be analyzed in further studies.

Another potential limitation of our study is that we documented the presence of TTSS genes but did not evaluate whether the corresponding cytotoxins are actually secreted in vitro. Indeed, it has been demonstrated that only a fraction of isolates, ranging from 44% to 77% according to different studies [16–18], secrete TTSS cytotoxins in vitro; further studies should therefore comparatively analyze the usefulness of TTSS genotype vs phenotype as prognostic marker of PA infections.

In conclusion, using a large multicenter prospective cohort of PA bacteremia, we demonstrate for the first time that the *exoU* genotype is independently associated with increased risk of early mortality of PA bloodstream infections, whereas late mortality is associated with MDR profiles. Our results also evidence a significant association between *exoU* genotype and individual resistance to several antipseudomonal agents, including carbapenems, fluoroquinolones, and cephalosporins. Finally, our results also highlight that XDR profiles are linked to a very limited number of specific clones (high-risk clones) presenting defined TTSS genotypes, which must be considered in the analysis of the infection outcomes.

Notes

Acknowledgments. We thank the other participants from the Spanish Network for Research in Infectious Diseases group: Mercedes Gurgui (Hospital Universitari de la Santa Creu i Sant Pau, Barcelona), Roger Sorde and Nieves Larrosa (Hospital Universitario Vall d'Hebrón) Cecilia Martín (Hospital Universitario Virgen del Rocío, Sevilla), Dionisia Fontanals (Consorci Hospitalari Parc Taulí, Sabadell, Barcelona), Marina de Cueto and Maria Dolores Navarro (Hospital Universitario Virgen Macarena, Sevilla), Julian Torre-Cisneros, Manuel Casal, Rosario Lara, Clara Natera and Antonio Rivero (Hospital Universitario Reina Sofía-IMIBIC, Córdoba).

Financial support. This work was supported by the Ministerio de Economía y Competitividad of Spain, Instituto de Salud Carlos III, cofinanced by European Regional Development Fund "A way to achieve Europe" through the Spanish Network for the Research in Infectious Diseases (RD06/0008 and RD12/0015) and grants 08/0276, PS09/00033, 11/00164, and PI12/00103. The study is also supported by Direcció General d'Universitats, Recerca i Transferència del Coneixement del Govern de les Illes Balears.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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