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Prospective Observational Study of Prior Rectal Colonization Status as a Predictor for Subsequent Development of *Pseudomonas aeruginosa* Clinical Infections

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The potential role of *Pseudomonas aeruginosa* (PA) intestinal colonization in the subsequent development of infections has not been thoroughly investigated. The aims of this study were to assess the role of PA intestinal colonization as a predictor of subsequent infections and to investigate the risk factors associated with the development of PA infection in patients in the intensive care unit (ICU). For this purpose, a prospective study was conducted that included (i) active surveillance of PA rectal colonization at ICU admission and weekly until ICU discharge, (ii) detection of PA clinical infections, and (iii) genotypic analysis by pulsed-field gel electrophoresis (PFGE). A total of 414 patients were included, of whom 179 (43%) were colonized with PA. Among the 77 patients who developed PA infection, 69 (90%) had prior PA colonization, and 60 (87%) of these showed genotyping concordance between rectal and clinical isolates. The probability of PA infection 14 days after ICU admission was 26% for carriers versus 5% for noncarriers ($P < 0.001$). Cox regression analysis identified prior PA rectal colonization as the main predictor of PA infection (hazard ratio [HR], 15.23; 95% confidence interval [CI], 6.9 to 33.7; $P < 0.001$). Prior use of nonantipseudomonal penicillins was also identified as an independent variable associated with PA infection (HR, 2.15; 95% CI, 1.3 to 3.55; $P < 0.003$). Our study demonstrated that prior PA rectal colonization is a key factor for developing PA infection.

Pseudomonas aeruginosa (PA) is a Gram-negative bacterium that is one of the most common nosocomial pathogens, causing severe infections with significant morbidity and mortality (1). PA has an intrinsic resistance to a wide range of antibiotics and a notable ability to acquire resistance during the course of antibiotic therapy, resulting in the development of multidrug-resistant strains (2). In recent decades, the incidence of infections caused by multidrug-resistant bacteria has continuously increased. This problem is of major concern due to the emergence of strains resistant to almost all of the available antimicrobial drugs (3).

Despite the growing number of antibiotic-resistant infections, the clinical consequences of multidrug resistance are still unclear. Experimental studies suggest a possible association between acquisition of resistance mechanisms and a fitness cost, which decreases the virulence of PA (4–6). Nevertheless, other researchers propose that resistant bacteria may develop additional compensatory mechanisms that can compensate for the fitness cost caused by resistant mutations (7, 8). The current data on the correlation between the resistance pattern of PA and clinical pathogenicity are limited. In an earlier study, our group determined the invasive capacity of PA by analyzing its ability to produce bloodstream infections (9). However, that study had some limitations, such as its retrospective design and the fact that no active surveillance was performed to detect PA colonization.

Intestinal colonization is believed to play an essential role in the pathogenesis of PA infection in patients in the intensive care unit (ICU) (10–14). Although it is widely assumed that colonization precedes the development of infection, little is known about its relative importance in this process. A few descriptive studies have been conducted to assess the issue (14–16); however, the design of these studies did not allow for an assessment of the temporal relationship. Genotype studies are needed to establish a causal link between surveillance strains and clinical samples.

We therefore conducted a prospective active surveillance program in ICU patients to investigate the role of PA rectal colonization as a predictor of subsequent PA infection to determine whether there are differences in the ability to develop infection based on the presence of PA on rectal surveillance cultures. We also investigated the potential influence of other variables on the development of PA infection in order to identify modifying risk factors associated with these infections.

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MATERIALS AND METHODS

Setting. This study was performed in one of the three general ICU units at the Hospital Universitari de Bellvitge, a 700-bed tertiary-care institution for adult patients in Barcelona. The ICU is a 12-bed, private-room ward

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providing care to medical and surgical patients, including those who have undergone solid-organ transplantation.

Study design and data collection. This prospective cohort study included all patients admitted to the ICU for >48 h during an 18-month period (1 January 2012 to 1 July 2013). The study design was approved by the Clinical Ethics Committee of the Hospital Universitari de Bellvitge, and patients or family provided written informed consent.

An active surveillance study was performed by obtaining rectal swab samples on ICU admission and weekly until ICU discharge in order to identify digestive tract colonization of PA. Patients were followed from ICU admission to ICU discharge in order to detect invasiveness (defined as the ability to develop infection in carriers) and to determine patients' outcomes. Clinical samples were collected as requested by medical staff.

Definitions. Demographic and clinical characteristics of each patient were collected prospectively. Sex, age, length of hospitalization before ICU admission, underlying diseases, and the Charlson comorbidity index as an indicator of patient comorbidity were recorded on admission (18). Patients were considered to have cancer when malignancy was diagnosed in the last 5 years or if they were receiving oncological therapy. They were considered to be immunosuppressed if chemotherapy, radiotherapy, corticosteroids, or other immunosuppressive agents were administered in the 3 months prior to the ICU admission. Prior surgery was defined as the presence of surgical events within 3 months prior to ICU admission. The Simplified Acute Physiologic Score (SAPS II) was used to estimate patients' disease severity at ICU admission (19). Prior hospital stay was defined as hospitalization in the last 3 months or hospital stay of >48 h before ICU admission. The source of infection was established according to the Centers for Disease Control and Prevention criteria (20). Ventilator-associated pneumonia (VAP) was defined as pneumonia that developed after the patient underwent intubation and received mechanical ventilation for at least 48 h. Ventilator-associated tracheobronchitis (VAT) is a clinical syndrome similar to VAP but with no radiographic infiltrate present (21); VAP and VAT were classified according to the clinical pulmonary infection score (CPIS). The diagnosis of VAP was made in the case of a CPIS of ≥6, while the diagnosis of VAT was made when the score was <6, requiring also the presence of clinical signs and symptoms (e.g., fever, purulent appearance of respiratory secretions, elevated leukocyte count) and microbiological criteria (i.e., growth of pathogens on a lower respiratory tract culture) (22). Ascertainment of the clinical infection was performed by the study investigators according to CDC definitions and CPIS score in cases of VAP/VAT.

Exposure to antibiotics was determined as the number of days of antibiotic treatment in the 3 months prior to the ICU admission and was recorded according to antibiotic family: fluoroquinolones, carbapenems, aminoglycosides, antipseudomonal, and nonantipseudomonal cephalosporins, and antipseudomonal and nonantipseudomonal penicillins. Data on other antibiotic families (colistin, glycopeptides, monobactam, and fosfomycin) were also collected, but no statistical analysis was performed because the mean length of exposure was very short (<1.5 days) and therefore of low relevance.

PA phenotypes were classified according to their pattern of antimicrobial resistance (23). Multidrug-resistant PA (MDR-PA) was defined as a strain nonsusceptible to at least one agent in three or more antipseudomonal antimicrobial categories. Extensively drug-resistant PA (XDR-PA) was defined as a strain nonsusceptible to at least one agent in all but two or fewer antipseudomonal antimicrobial categories. Therefore, XDR-PA strains were also included as MDR-PA. Non-multidrug-resistant PA (non-MDR-PA) was defined as a wild-type strain or a strain nonsusceptible to at least one agent in fewer than three antipseudomonal antimicrobial categories.

Microbiological studies. PA strains were identified and tested for antimicrobial susceptibility by a MicroScan automated microdilution system using CN1S and CO1S panels (Dade International, West Sacramento, CA, USA). CLSI criteria were used to define susceptibility or resistance to these antimicrobial agents (24). Pulsed-field gel electrophoresis (PFGE) analysis using a method described previously was performed to identify any relationship between the strain isolated in the rectal swab and the subsequent clinical sample from the source of infection (25). We performed PFGE analysis of 69 pairs of PA strains isolated from the same patient: 42 samples of non-MDR, 12 pairs of MDR non-XDR, and 15 pairs of XDR. DNA restriction patterns generated by PFGE were interpreted according to the guidelines (26).

Statistical analysis. Comparative analyses of baseline characteristics of the groups based on results of surveillance culture were performed with the Student *t* test or the Mann-Whitney *U* test for continuous variables and the χ^2 or Fisher's exact test for categorical variables, as appropriate (Table 1). Continuous variables are expressed as means (standard deviation) or medians (interquartile range), depending on the distribution.

The Kaplan-Meier method was used to investigate the role of PA rectal colonization in subsequent infections. Only single patients were included. PA clinical infection was considered the main event. Time zero was the date of ICU admission, and patients were censored when they were discharged from the ICU or when they died for reasons other than infection. The risk to develop clinical infections due to PA is different before and after a prior rectal colonization. Therefore, positive surveillance culture was treated as a time-dependent variable to deal with the difference in risk. The time-dependent covariate was created using the date of the first positive surveillance culture. Univariate and multivariate analyses of parameters predicting PA infection were made with Cox regression analysis (Table 2). Variables that were associated with infection and had a *P* value of <0.10 in the crude analyses were included in the adjusted analysis. To compare the ability to develop infection according to the profile of resistance, we included only the first episode of infection. If colonization was demonstrated after infection, or if no rectal colonization was identified during the follow-up, the episode of infection was not analyzed (Fig. 1).

The impact of prior antimicrobial therapy was evaluated when comparing the rectal colonization status of the patients (Table 1). To do that, data on antibiotic exposure were collected from 3 months prior to ICU admission until the day that surveillance culture become positive in colonized patients or the day of discharge or death in noncolonized patients. In regard to analyses conducted to identify parameters associated with PA clinical infection (Table 2), antibiotic exposure was measured until the day of the infection for infected patients and until the day patients were censored (discharge or death) if infection did not occur. Antibiotic exposure was analyzed as a binary variable and was recorded according to antibiotic classes. Data were analyzed using SPSS (Statistical Package for Social Sciences) version 19.0 and R version 3.1.2 software. A *P* value of <0.05 indicated statistical significance.

RESULTS

Epidemiological and clinical characteristics. A total of 414 patients were included in the study, of whom 179 (43%) were colonized with PA. Information describing the study sample was reported elsewhere (27).

During the study, 97 episodes of PA infection occurred in 77 patients: 14 patients presented two episodes of PA infection, and three patients presented three episodes. The overall incidence of PA infection in ICU patients during the study was 19% (77/414). Clinical and epidemiological characteristics of the patients included in the study are shown in Table 1. Among the 77 first episodes of infection, 45 (58%) were caused by non-MDR-PA, 12 (16%) by MDR non-XDR strains, and 20 (26%) by XDR-PA. The source of the infection in non-MDR-PA infections was respiratory in 34 patients (76%), intravascular catheter-related bacteremia in 3 (7%), intra-abdominal in 2 (4%), osteoarticular in 1 (2%), and other sources in 5 (11%), 1 of whom had bacteremia. The source of MDR non-XDR infection was respiratory in 10 (84%) patients (2 with bloodstream infection), intravascular catheter-related bacteremia in 1 (8%), and intra-abdominal in 1 (8%). The origin

TABLE 1 Clinical and epidemiological characteristics of patients based on results of surveillance culture

Variable	No. of PA[a] rectal colonized patients (%) ($n = 179$)	No. of PA noncolonized patients (%) ($n = 235$)	*P* value
Baseline characteristics			
Age (yr, median [interquartile range])	66. 8 (56.4–75.3)	64.3 (51.2–73.1)	0.06
Sex, male	126 (70)	139 (59)	0.013
Charlson index score (median [interquartile range])	2 (1–4)	2 (1–4)	0.76
SAPS II score at ICU admission (median [interquartile range])	44 (36–52)	43 (34–51)	0.46
Main underlying disease			
Diabetes	43 (24)	65 (28)	0.50
Chronic pulmonary disease	47 (26)	47 (20)	0.13
Cardiovascular disease	75 (42)	75 (32)	0.04
End-stage renal failure	24 (13)	30 (13)	0.88
Chronic liver disease	24 (13)	30 (13)	0.88
Vascular/degenerative brain disease	25 (9)	20 (14)	0.08
Malignant disease	34 (19)	55 (23)	0.34
Immunosuppressed	44 (25)	63 (27)	0.73
Prior hospital stay	132 (73)	96 (41)	<0.001
Prior surgery	107 (60)	125 (53)	0.16
Hospital mortality	54 (30)	65 (28)	0.58
Prior antibiotic use (days, median [interquartile range])	7 (2–14)	8 (0–16)	0.95
Fluoroquinolones	35 (20)	41 (17)	0.61
Carbapenems	46(26)	60 (26)	1
Antipseudomonal penicillins	68 (38)	70 (30)	0.07
Nonantipseudomonal penicillins	65 (36)	57 (24)	0.007
Antipseudomonal cephalosporins	16 (8)	16 (7)	0.46
Nonantipseudomonal cephalosporins	20 (11)	22 (9)	0.62
Aminoglycosides	5 (3)	6 (3)	0.93
Source of PA clinical infection	71 (92)	6 (8)	<0.001
Respiratory[b]	53 (69)	3 (4)	
VAP	38 (49)	1 (1)	
VAT	15 (20)	2 (3)	
Urinary	2 (3)	0 (0)	
Catheter	6 (8)	2 (3)	
Intra-abdominal	3 (4)	1 (1)	
Osteoarticular	1 (1)	0 (0)	
Unknown/endogenous	1 (1)	0 (0)	
Other	5 (6)	0 (0)	

[a] PA, *Pseudomonas aeruginosa*.
[b] VAP, ventilator-associated pneumonia; VAT, ventilator-associated tracheobronchitis.

of XDR-PA infection was respiratory in 12 (60%) patients (1 with bacteremia), intravascular catheter-related bacteremia in 4 (20%), urinary-source bacteremia in 2 (10%), intra-abdominal in 1 (5%), and unknown-source bacteremia in 1 (5%). XDR and other PA phenotype strains differed significantly in regard to the source of infection, with XDR-PA infection presenting a higher percentage with a vascular or urinary catheter focus (6 patients [30%] in XDR-PA versus 4 patients [7%] in non-XDR-PA; $P = 0.016$).

Phenotypic and genotypic analysis. Among the 69 pairs of PA strains with subsequent infection, 60 (87%) showed concordance between rectal and clinical isolates: 39/42 (93%) in non-MDR infections, 8/12 (67%) in MDR non-XDR infections, and 13/15 (87%) in XDR infections. The genotypic analysis confirmed a clonal dissemination in XDR-PA strains, which was due to a cluster belonging to the high-risk clone ST175.

Risk factors for PA infection. Among the 77 patients who developed PA infection, 69 (90%) presented with prior PA intestinal colonization, while 8 (10%) did not (5 patients with no colonization during admission and 3 with rectal colonization identified after PA infection) (Fig. 1). The proportion of infection was 39% in colonized patients and 3.4% in noncolonized patients ($P < 0.001$).

The unadjusted probabilities of PA infection in PA-colonized patients are shown in Fig. 2. The probability of PA infection at 14 days after ICU admission was 26% for PA-colonized patients versus 5% for noncolonized patients (log rank, $P < 0.001$). Baseline characteristics and variables examined as possible predictors of PA clinical infection are displayed in Table 2. After adjusting for Charlson index, chronic liver disease, prior PA rectal colonization, and prior consumption of fluoroquinolones, antipseudomonal penicillins, nonantipseudomonal penicillins, and nonantipseudomonal cephalosporins, a Cox regression model showed prior PA intestinal colonization to be the main factor associated with development of PA infection (hazard ratio [HR], 15.23; 95% confidence interval [CI], 6.9 to 33.7; $P < 0.001$).

Sixty first episodes of infection were included (39 non-MDR, 8 MDR non-XDR, and 13 XDR) (Fig. 1). Due to the existence of a

TABLE 2 Cox regression analysis of predictive factors for the development of clinical infections due to *P. aeruginosa*

Variable[a]	No. with clinical PA infection (%) ($n = 77$)	No. with nonclinical PA infection (%) ($n = 337$)	Crude analysis		Adjusted analysis	
			HR (95% CI)	*P* value	HR (95% CI)	*P* value
Baseline feature						
Age >65 yr	40 (52)	175 (52)	1.02 (0.65–1.62)	0.92		
Male	57 (74)	208 (62)	0.88 (0.68–1.13)	0.31		
Charlson score >2	40 (52)	151 (45)	1.49 (0.95–2.34)	0.08	1.18 (0.7–2.01)	0.531
SAPS II >40 score at ICU admission	45 (58)	195 (58)	1.01 (0.63–1.59)	0.98		
Main underlying disease						
Diabetes	14 (18)	94 (28)	0.85 (0.47–1.52)	0.58		
Chronic pulmonary disease	17 (22)	77 (23)	1.05 (0.61–1.80)	0.86		
Cardiovascular disease	26 (34)	124 (37)	1.02 (0.63–1.63)	0.95		
End-stage renal failure	7 (9)	47 (14)	0.93 (0.43–2.05)	0.86		
Chronic liver disease	14 (18)	40 (12)	1.81 (1.01–3.24)	0.046	1.52 (0.77–3.01)	0.228
Vascular/degenerative brain disease	11 (14)	34 (10)	0.95 (0.50–4.82)	0.89		
Malignant disease	17 (22)	72 (21)	0.87 (0.50–1.53)	0.64		
Immunosuppressed	21 (27)	86 (26)	0.78 (0.56–1.55)	0.78		
Prior hospital stay	72 (94)	156 (46)	10.1 (4.08–25)	<0.001		
Prior surgery	49 (64)	182 (54)	1.28 (0.80–2.06)	0.29		
Hospital mortality	26 (34)	93 (28)	1.41 (0.87–2.28)	0.16		
Prior rectal colonization	69 (90)	107 (32)	16.57 (7.53–36.49)	<0.001	15.23 (6.9–33.7)	<0.001
Prior antibiotic use >10 days	41 (53)	155 (41)	1.33 (0.80–2.12)	0.27		
Fluoroquinolones	12 (16)	74 (22)	0.42 (0.22–0.78)	0.006	0.40 (0.21–0.76)	0.005
Carbapenems	25 (32)	96 (28)	0.76 (0.48–1.26)	0.30		
Antipseudomonal penicillins	29 (38)	128 (38)	0.33 (0.20–0.54)	<0.001	0.39 (0.23–0.64)	<0.001
Nonantipseudomonal penicillins	34 (44)	88 (26)	1.71 (1.08–2.70)	0.022	2.15 (1.3–3.55)	0.003
Antipseudomonal cephalosporins	11 (15)	25 (7)	1.35 (0.71–2.57)	0.36		
Nonantipseudomonal cephalosporins	17 (22)	31 (9)	1.88 (1.08–3.27)	0.026	1.05 (0.58–1.9)	0.865
Aminoglycosides	3 (4)	8 (2)	1.01 (0.32–3.22)	0.99		

[a] For the multivariate analyses, variables with a *P* value of <0.10 in the univariate analyses were included. To avoid colinearity problems, the final model does not include the prior hospital stay variable as it is nearly predicted by the prior colonization variable. Differential risks for development of prior rectal colonization were accounted for using a time-dependent variable within the model. Abbreviations: PA, *Pseudomonas aeruginosa*; HR, hazard ratio; CI, confidence interval.

clonal dissemination in XDR-PA strains and the different behavior of this cluster, we only analyzed differences in the ability to produce infection in polyclonal strains. The probability of infection at 14 days of colonization was 26% for non-MDR versus 16% for MDR non-XDR (log rank, $P = 0.13$). After adjusting to avoid potential confounding factors in a multivariate analysis, a trend toward an increased risk of infection in non-MDR-PA intestinal carriers was observed (HR, 1.75; 95% CI, 0.81 to 3.8; $P = 0.15$).

DISCUSSION

We designed a prospective study of a 1.5-year cohort of patients admitted to a medical and surgical ICU in order to investigate whether PA intestinal colonization contributes to the development of PA infection acquired in the ICU. Our study demonstrated a significant association between PA-colonized patients and the development of PA infection, as evidenced by the fact that the risk of developing PA clinical infections among PA-colonized patients was 15 times higher than the risk found in noncolonized patients (HR, 15.23; 95% CI, 6.9 to 33.7; $P < 0.001$).

PA has a remarkable ability to colonize and infect patients. Classic epidemiological analysis suggests that there are two major routes of colonization: endogenous and exogenous (10, 11, 13). Intestinal colonization is considered the most important endogenous source preceding the development of PA infection. In these cases, PA infections are polyclonal and caused by the patient's own flora after PA pathological colonization of endogenous reservoirs (10, 13, 14). Additionally, exogenous routes have been demonstrated to play an essential role in the pathogenesis of PA infection, mainly in outbreak situations (13). In the exogenous colonization, patients may become colonized from contaminated reservoirs or other colonized patients (cross-colonization) (13, 14).

Previous studies of digestive colonization have shown that patients who are colonized are significantly more likely to have episodes of invasive infection than patients who are not. The average rate of infection in colonized patients ranges from 15% to 90%. This discrepancy in the rate of invasive infection in the few studies published may be due in part to the patients' conditions and the methodology applied (10, 14, 16). The sample size in one of these studies was very small, and the correlation between rectal colonization and development of infection was not definitive, since neither serial intestinal tract cultures nor genotypic studies were performed (14).

Thus, to evaluate the association between colonization and infection, we conducted a weekly active surveillance of intestinal tract PA colonization to establish the temporal relation, and we did a molecular study of the strains to define the genetic relatedness of surveillance and clinical samples. Depending on the presence or absence of prior rectal colonization, differences in the ability to develop PA infection were found. In the multivariate model, previous PA intestinal colonization was the main risk factor for the development of PA infection.

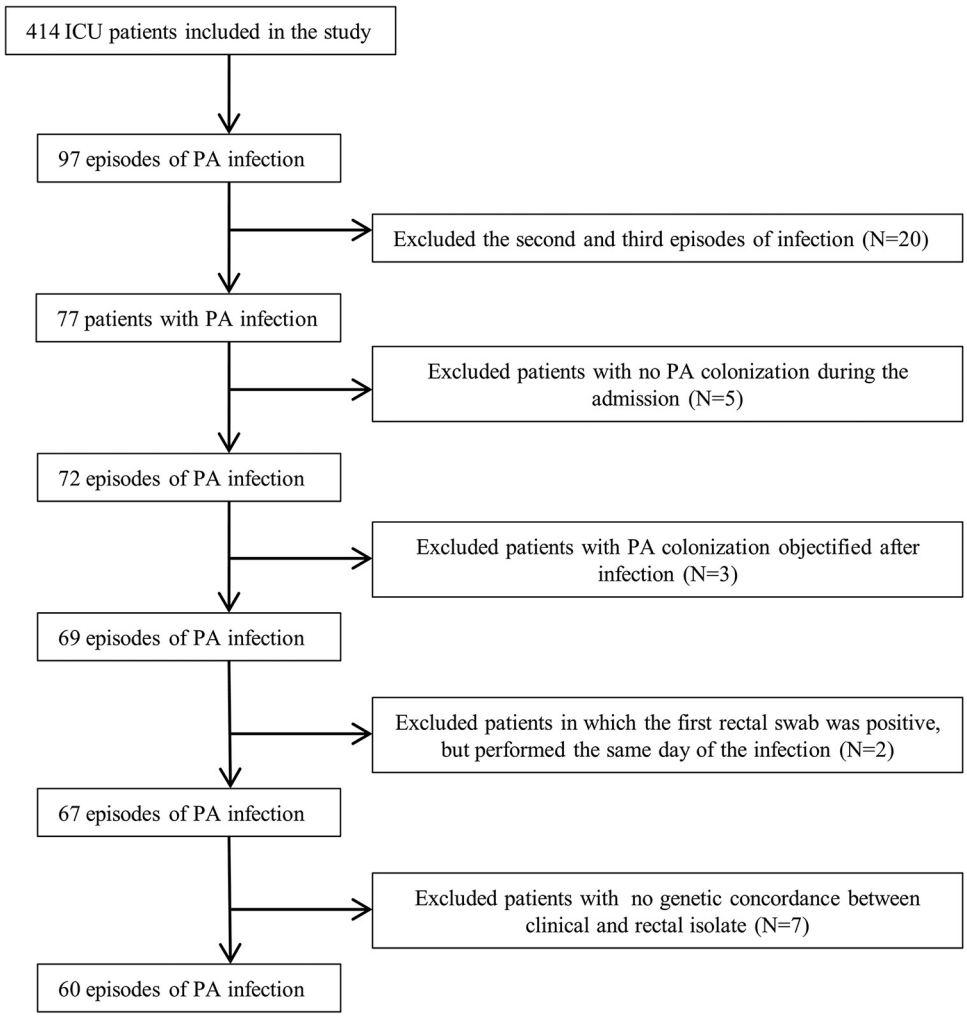

FIG 1 Flow diagram of *Pseudomonas aeruginosa* (PA) clinical infection selection process, indicating episode.

A retrospective cohort study was done in an ICU where surveillance for detecting Gram-negative bacteria was performed routinely. Researchers found that 74.5% of bacteremia incidences were preceded by colonization, and they were associated with higher rates of appropriate therapy than those occurring in noncolonized patients (12). In line with our findings, a recent retrospective cohort study showed that the presence of carbapenem-resistant *Acinetobacter baumannii* on surveillance cultures was strongly associated with the development of carbapenem-resistant *A. baumannii* infections (28).Our study supports these data, as the genotype study revealed an 87% concordance between surveillance and clinical samples. These molecular data, together with the greater ability to develop infection in patients previously colonized with PA (39%), strongly suggest that PA intestinal colonization is a key requirement for developing PA infection in ICU patients. Moreover, knowledge of PA colonization may help to initiate appropriate empirical therapy and may make it possible to restrict the use of the limited number of drugs available for treating MDR-PA infections (colistin or amikacin) to a select group of patients, thus avoiding the deleterious effect of these drugs in patients not receiving these drugs.

Interestingly, the molecular analysis study of pairs did not coincide in three patients with surveillance and clinical samples presenting non-MDR-PA strains. We cannot dismiss the possibility that multiple genotypes of non-MDR-PA strains were present in rectal samples and were not detected due to the impossibility of isolating all strains with a similar phenotype. This difficulty may also have appeared in the six noncoincident pairs of MDR phenotypes. This underlines the complexity of the clinical epidemiology of PA and the difficulty in drawing conclusions regarding epidemiological causality without molecular studies.

Molecular analysis demonstrated the existence of a clonal dissemination in XDR PA strains, which was responsible for an endemic outbreak in our hospital that was previously described (29). The epidemic nature of the XDR-PA cluster may explain differences in the sources of infection, the most common in XDR-PA infection being the vascular and urinary catheter focused, which are associated with a relatively high rate of manipulation and subsequent horizontal transmission. When we compared the ability of polyclonal non-MDR and polyclonal MDR strains to develop infection, we found a trend toward an increased risk of infection in patients colonized by non-MDR-PA strains, which may suggest that non-MDR strains are more pathogenic. However, these differences are not significant, probably due to the small sample size,

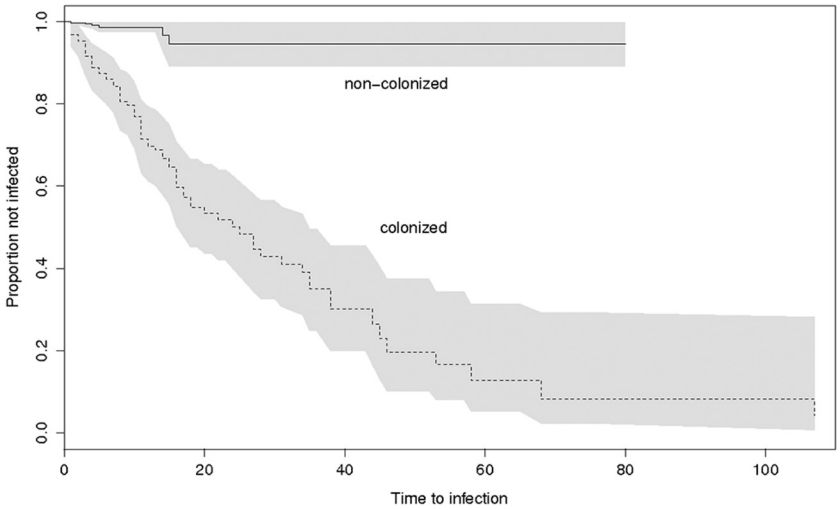

FIG 2 Survival curves showing the probability of developing *Pseudomonas aeruginosa* (PA) infection based on rectal colonization status. The start of the risk period was defined as the ICU admission. Solid line, noncolonized patients; dashed line, PA-colonized patients. The areas of 95% confidence intervals of both groups are shown.

and large future studies are needed to confirm the interplay between antibiotic resistance and pathogenicity in the clinical setting.

Finally, previous studies assessed the influence of antimicrobial use in PA intestinal colonization (27). However, little is known about the role of antibiotic therapy in the development of invasive PA infection. In our study, nonantipseudomonal penicillins were associated with PA infection. PA is a microorganism with inherent resistance to these antibiotic classes, and their consumption in PA-colonized patients may lead to selection of PA, thus causing collateral damage to the endogenous microflora (10, 14, 16). While, overall, antibiotics play a decisive role in selecting intestinal flora, fluoroquinolones and antipseudomonal penicillins can preserve activity against a significant number of PA strains and thus protect against the development of PA infections.

The current study is the largest prospective analysis of the role of PA intestinal colonization in subsequent infection. However, several limitations should be mentioned. Despite being a large cohort, the data set in which we performed our analysis of invasiveness according to the resistance profile of PA was limited by the small number of patients infected by different phenotypes. Moreover, the presence of an epidemic clone with different epidemiological behavior forced us to reduce the sample further. Finally, medical staff were not blinded to the rectal surveillance status.

To our knowledge, this is the first study to focus on the ability of PA to develop infection among PA-colonized patients by means of serial active surveillance and genotypic studies. It demonstrates definitively that PA intestinal colonization is a key requirement for the development of PA infection in ICU patients. Additional epidemiological studies are needed to confirm the implications of resistance in clinical invasiveness and to guide antibiotic policy by allowing a more judicious use of the few antimicrobial options available.

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REFERENCES

1. **Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, Kallen A, Limbago B, Fridkin S, National Healthcare Safety Network (NHSN) Team and Participating NHSN Facilities.** 2013. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol **14:**1–14. http://dx.doi.org/10.1086/668770.
2. **Livermore DM.** 2002. Multiple mechanism of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare. Clin Infect Dis **34:**634–640. http://dx.doi.org/10.1086/338782.
3. **Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J.** 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis **48:**1–12. http://dx.doi.org/10.1086/595011.
4. **Abdelraouf K, Kabbara S, Ledesma KR, Poole K, Tam VH.** 2011. Effect of multidrug resistance-conferring mutations on fitness and virulence of *Pseudomonas aeruginosa*. J Antimicrob Chemother **66:**1311–1317. http://dx.doi.org/10.1093/jac/dkr105.
5. **Sun Z, Jiao X, Peng Q, Jiang F, Huang Y, Zhang J, Yao F.** 2013. Antibiotic resistance in *Pseudomonas aeruginosa* is associated with decreased fitness. Cell Physiol Biochem **31:**347–354. http://dx.doi.org/10.1159/000343372.
6. **Giamarellos-Bourboulis EJ, Plachouras D, Tzivra A, Kousoulas V, Bolanos N, Raftogiannis M, Galani I, Dontas I, Dionyssiou-Asteriou A, Giamerellou H.** 2004. Simulation of innate immunity by susceptible and multidrug-resistant *Pseudomonas aeruginosa*: an *in vitro* and *in vivo* study. Clin Exp Immunol **135:**240–246. http://dx.doi.org/10.1111/j.1365-2249.2003.02365.x.
7. **Kugelberg E, Löfmark S, Wretlind B, Andersson DI.** 2005. Reduction of the fitness burden of quinolone resistance in *Pseudomonas aeruginosa*. J Antimicrob Chemother **55:**22–30. http://dx.doi.org/10.1093/jac/dkh505.
8. **Andersson DI, Hughes D.** 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol **8:**260–271. http://dx.doi.org/10.1037/nrmicro2319.
9. **Peña C, Gómez-Zorrilla S, Suárez C, Dominguez MA, Tubau F, Arch O, Oliver A, Pujol M, Ariza J.** 2012. Extensively drug-resistant *Pseudomonas*

aeruginosa: risk of bloodstream infection in hospitalized patients. Eur J Clin Microbiol Infect Dis **31:**2791–2797. http://dx.doi.org/10.1007/s10096-012-1629-3.

10. **Bonten MJ, Bergmans DC, Speijer H, Stobberingh EE.** 1999. Characteristic of polyclonal endemicity of *Pseudomonas aeruginosa* colonization in intensive care units. Am J Respir Crit Care Med **160:**1212–1219. http://dx.doi.org/10.1164/ajrccm.160.4.9809031.
11. **Bertrand X, Thouverez M, Talon D, Boillot A, Capellier G, Floriot C, Hélias JP.** 2001. Endemicity, molecular diversity and colonization routes of *Pseudomonas aeruginosa* in intensive care units. Intensive Care Med **27:**1263–1268. http://dx.doi.org/10.1007/s001340100979.
12. **Blot S, Depuydt P, Vogelaers D, Decruyenaere J, De Waele J, Hoste E, Peleman R, Claeys G, Verschraegen G, Colardyn F, Vandewoude K.** 2005. Colonization status and appropriate antibiotic therapy for nosocomial bacteremia caused by antibiotic-resistant gram-negative bacteria in an intensive care unit. Infect Control Hosp Epidemiol **26:**575–579. http://dx.doi.org/10.1086/502575.
13. **Peña C, Guzmán A, Suarez C, Dominguez MA, Tubau F, Pujol M, Gudiol F, Ariza J.** 2007. Effects of carbapenem exposure on the risk for digestive tract carriage on intensive care unit-endemic carbapenem-resistant *Pseudomonas aeruginosa* strains in critically ill patients. Antimicrob Agents Chemother **51:**1967–1971. http://dx.doi.org/10.1128/AAC.01483-06.
14. **Marshall JC, Christou NV, Meakins JL.** 1993. The gastrointestinal tract: the "undrained abscess" of multiple organ failure. Ann Surg **218:**111–119. http://dx.doi.org/10.1097/00000658-199308000-00001.
15. **Noone MR, Pitt TL, Bedder M, Hewlett AM, Rogers KB.** 1983. *Pseudomonas aeruginosa* colonization in an intensive therapy unit: role of cross infection and host factors. Br Med J (Clin Res Ed) **286:**341–344. http://dx.doi.org/10.1136/bmj.286.6362.341.
16. **Ohara T, Itoh K.** 2003. Significance of *Pseudomonas aeruginosa* colonization of the gastrointestinal tract. Intern Med **42:**1072–1076. http://dx.doi.org/10.2169/internalmedicine.42.1072.
17. **Gómez-Zorrilla S, Camoez M, Tubau F, Cañizares R, Periche E, Dominguez MA, Ariza J, Peña C.** 2014. Clinical invasiveness on patients with multidrug-resistant (MDR) and non-MDR *Pseudomonas aeruginosa* (PA) colonization, abstr K1047. Abstr 54th Intersci Conf Antimicrob Agents Chemother, Washington, DC, 5 to 9 September 2014.
18. **Charlson ME, Pompei P, Ales KL, MacKenzie CR.** 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis **40:**373–383. http://dx.doi.org/10.1016/0021-9681(87)90171-8.
19. **Le Gall JR, Loirat P, Alperovitch A, Glaser P, Granthil C, Mathieu D, Mercier P, Thomas R, Villers D.** 1984. A simplified acute physiology score for ICU patients. Crit Care Med **12:**975–977. http://dx.doi.org/10.1097/00003246-198411000-00012.
20. **Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM.** 1988. CDC definitions for nosocomial infections, 1988. Am J Infect Control **16:**128–140. http://dx.doi.org/10.1016/0196-6553(88)90053-3.
21. **Craven DE; Hialmarson KI.** 2010. Ventilator-associated tracheobronchitis and pneumonia: thinking outside the box. Clin Infect Dis **51**(Suppl 1):S59–S66. . http://dx.doi.org/10.1086/653051.
22. **Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM.** 1991. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. Am Rev Respir Dis **143:**1121–1129. http://dx.doi.org/10.1164/ajrccm/143.5_Pt_1.1121.
23. **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske GK, Harbarth S, Hindler JF, Kahimeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL.** 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **18:**268–281. http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x.
24. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. CLSI M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.
25. **Deplano A, Denis O, Poriel L, Hocquet D, Nonhoff C, Byl B, Nordmann P, Vincent JL, Struelens MJ.** 2005. Molecular characterization of an epidemic clone of panantibiotic-resistant *Pseudomonas aeruginosa*. J Clin Microbiol **43:**1198–1204. http://dx.doi.org/10.1128/JCM.43.3.1198-1204.2005.
26. **Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **33:**2233–2239.
27. **Gómez-Zorrilla S, Camoez M, Tubau F, Periche E, Cañizares R, Dominguez MA, Ariza J, Peña C.** 2014. Antibiotic pressure is a major risk factor for rectal colonization by multidrug-resistant *Pseudomonas aeruginosa* in critically ill patients. Antimicrob Agents Chemother **58:**5863–5870. http://dx.doi.org/10.1128/AAC.03419-14.
28. **Latibeaudiere R, Rosa R, Laowansiri P, Arheart K, Namias N, Munoz-Price LS.** 2015. Surveillance cultures growing carbapenem-resistant *Acinetobacter baumannii* predict the development of clinical infections: a retrospective cohort study. Clin Infect Dis **60:**415–422. http://dx.doi.org/10.1093/cid/ciu847.
29. **Suarez S, Peña C, Arch O, Dominguez MA, Tubau F, Juan C, Gavaldá L, Sora M, Oliver A, Pujol M, Ariza J.** 2011. A large sustained endemic outbreak of multiresistant *Pseudomonas aeruginosa*: a new epidemiological scenario for nosocomial acquisition. BMC Infect Dis **11:**272. http://dx.doi.org/10.1186/1471-2334-11-272.