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José A. Martínez
Esther Delgado
Sara Martí
Francesc Marco
Jordi Vila
Josep Mensa
Antoni Torres
Carles Codina
Antoni Trilla
Alex Soriano
Aitor Alquezar
Pedro Castro
José M. Nicolás

Influence of antipseudomonal agents on *Pseudomonas aeruginosa* colonization and acquisition of resistance in critically ill medical patients

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J. A. Martínez (✉) · J. Mensa · A. Soriano
Department of Infectious Diseases, Hospital Clínic, IDIBAPS, University of Barcelona, Villarroel 170, 08036 Barcelona, Spain
e-mail: jamarti@clinic.ub.es
Tel.: +34-932272322
Fax: +34-934514438

E. Delgado · A. Alquezar · P. Castro · J. M. Nicolás
Medical Intensive Care Unit, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

S. Martí · F. Marco · J. Vila
Microbiology Laboratory, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

A. Torres
Respiratory Intensive Care Unit-ICPTC, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

C. Codina
Department of Pharmacy, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

A. Trilla
Unit for Evaluation, Supporting and Prevention, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona, Spain

Abstract *Objective:* To assess the role of antipseudomonal agents on *Pseudomonas aeruginosa* colonization and acquisition of resistance. *Design:* Prospective cohort study. *Setting:* Two medical intensive care units. *Patients and participants:* 346 patients admitted for ≥ 48 h. *Intervention:* Analysis of data from an 8-month study comparing a mixing versus a cycling strategy of antibiotic use. *Measurements and results:* Surveillance cultures from nares, pharynx, rectum, and respiratory secretions were obtained thrice weekly. Acquisition of resistance was defined as the isolation, after 48 h of ICU stay, of an isolate resistant to a given antibiotic if culture of admission samples were either negative or positive for a susceptible isolate. Emergence of resistance refers to the conversion of a defined pulsotype from susceptible to non-susceptible. Forty-four (13%) patients acquired 52 strains of *P. aeruginosa*. Administration of piperacillin-tazobactam for ≥ 3 days (OR 2.6, 95% CI 1.09–6.27) and use of amikacin for ≥ 3 days (OR 2.6, 95% CI 1.04–6.7) were positively associated with acquisition of *P. aeruginosa*, whereas use of quinolones (OR 0.27, 95% CI 0.1–0.7) and antipseudomonal cephalosporins (OR 0.27, 95% CI 0.08–0.9) was protective. Exposure to quinolones and cephalosporins was not associated with the acquisition of resistance, whereas it was linked with usage of all other agents. Neither quinolones nor cephalosporins were a major determinant on the emergence of resistance to themselves, as resistance to these antibiotics developed at a similar frequency in non-exposed patients. *Conclusions:* In critically ill patients, quinolones and antipseudomonal cephalosporins may prevent the acquisition of *P. aeruginosa* and may have a negligible influence on the acquisition and emergence of resistance.

Keywords Intensive care unit · *Pseudomonas aeruginosa* · Antipseudomonal agents · Antimicrobial resistance · Emergence of resistance · Nosocomial infection

Introduction

Pseudomonas aeruginosa is a leading cause of nosocomial infections in critically ill patients, and clinical disease is usually preceded by the settlement of the pathogen on mucosal surfaces [1]. Previous studies have established that acquisition of *P. aeruginosa* is associated with the administration of antimicrobial agents devoid of antipseudomonal activity [2, 3]. In this regard, however, the role of antipseudomonal agents is less clear. Some studies which have linked prior use of antibiotics with ventilator-associated pneumonia due to *P.* aeruginosa [4] or potentially resistant organisms (essentially *P. aeruginosa*, other non-fermenters and methicillin-resistant *S. aureus*) [5] have suggested that certain drugs such as imipenem, aminoglycosides and fluoroquinolones may have a role as individual risk factors, although their specific contribution has never been assessed.

P. aeruginosa is remarkable by the spontaneous generation of mutants resistant to any currently used antipseudomonal agent, which may be selected under appropriate antibiotic exposure. Emergence of resistance has been reported to occur in 6–53% of patients treated with any antipseudomonal agent [6–8]. In addition, several cohort or case-control studies have also linked previous use of antipseudomonal agents with the acquisition of *P. aeruginosa* resistant to the corresponding antibiotic [3, 9–21], other agents or multiple drugs [10, 12, 13, 15, 19, 22–31].

During an intervention study aimed to compare a mixing versus a cycling strategy of antibiotics use in the critical care setting [32], we were able to gather detailed longitudinal data about exposure to antibiotics and colonization by *P. aeruginosa.* We hypothesized that the risk of acquiring *P. aeruginosa*, of ending up by carrying a strain resistant to a given antipseudomonal agent that was susceptible or not present on admission and of developing of resistance in previously susceptible strains, could vary according to the particular antimicrobial to which the patient was exposed. The aims of the present study were to assess the role that exposure to each one of the antipseudomonal agents could have on the acquisition of *P. aeruginosa*, the role of exposure on the eventual carriage of a strain resistant to the same or a different antipseudomonal agent, and the role of exposure on the emergence of resistance in patients previously colonized with a susceptible strain.

Patients and methods

During eight consecutive months (from 15 October 2001 to 15 June 2002), patients admitted to two ICUs (a eight-bed medical ICU and a respiratory ICU with six beds) of a 700-bed university hospital whose expected length of stay was at least 2 days were included in a study aimed to compare the influence of two strategies (mixing vs. cycling) of use of anti-*Pseudomonas* antibiotics on the acquisition of resistant Gram-negative bacilli [32]. Patients were assigned to receive a cephalosporin, ciprofloxacin, a carbapenem or piperacillin-tazobactam in this order. Cycling was accomplished by prescribing one of these antibiotics during 1-month each. Mixing was accomplished by using the same order of antibiotic administration on consecutive patients. Interventions were carried out during two successive 4-month periods, starting with mixing in one unit and cycling in the other.

Microbiological procedures

Swabbing of nares, pharynx and rectum, and culture of respiratory secretions (tracheobronchial aspirates or sputum) were obtained thrice weekly. In addition, clinical samples were obtained as deemed necessary by the attending physician. No environmental cultures were taken. Susceptibility testing was done by a microdilution technique according to the CLSI guidelines [33]. For the purpose of analysis, intermediate susceptibility was considered as resistance. Molecular typing was performed by pulse-field gel electrophoresis (PFGE) using *SpeI* endonuclease as previously described [34].

Clinical variables

The set of clinical variables recorded is described in detail elsewhere [32] and correspond to those showed in Table 1. For the purposes of analysis, ceftazidime and cefepime were grouped as cephalosporins, imipenem and meropenem as carbapenems, and ciprofloxacin and levofloxacin as quinolones. Piperacillin-tazobactam and amikacin were considered individually.

Definitions

Patients with positive cultures within 48 h of admission to the units were considered to be colonized on admission. Microorganisms isolated after 48 h of admission were considered as ICU-acquired. In regards to species, acquisition was defined as a positive culture after 48 h of admission with previous negative specimens. Patients who acquired a pulsotype identical to that of an isolate previously found in another patient staying in the same unit and in the same period were considered to be cases of cross-transmission. Acquisition of resistance was defined as the isolation, after 48 h of ICU stay, of an isolate resistant to a given antimicrobial agent if culture of admission samples were either negative or positive for a susceptible isolate. Emergence of resistance to a given

Table 1 Relation of patient characteristics and previous exposures with acquisition of *P. aeruginosa*

Characteristic	Patients acquiring *P. aeruginosa* ($n = 44$)	Patients not acquiring *P. aeruginosa* ($n = 302$)	*P*
On ICU admission			
Age, mean ± SD	61 ± 17	63 ± 15	0.4
Male gender	30 (68)	190 (63)	0.5
Length of stay in hospital, median (IQ range)	3 (1–8)	5 (2–11)	0.4
Any prior antibiotic	7 (16)	76 (25)	0.2
Prior corticosteroids	8 (18)	26 (9)	0.06
APACHE II score, mean ± SD	17.5 ± 6	16.1 ± 6	0.16
SOFA score, mean ± SD	8.38 ± 2.2	7.6 ± 2.7	0.09
Surgery	12 (27)	79 (26)	0.8
Shock	19 (43)	66 (22)	0.002
Heart failure	4 (9)	12 (4)	0.13
COPD	8 (18)	66 (22)	0.6
Neoplasia	3 (7)	47 (16)	0.2
Liver cirrhosis	0	14 (5)	0.2
During ICU stay			
Time at risk, median (IQ range)	6 (4.25–11.5)	5 (3–9)	0.01
Mixing period	26 (59)	153 (51)	0.3
Cephalosporins	4 (9)	66 (22)	0.05
Cephalosporins ≥ 3 days	3 (7)	44 (15)	0.2
Carbapenems	11 (25)	70 (23)	0.8
Carbapenems ≥ 3 days	10 (23)	50 (17)	0.3
Quinolones	7 (16)	97 (32)	0.03
Quinolones ≥ 3 days	5 (11)	71 (24)	0.06
Piperacillin-tazobactam	18 (41)	51 (17)	0.0002
Piperacillin-tazobactam ≥ 3 days	17 (39)	31 (10)	<0.0001
Amikacin	14 (32)	50 (17)	0.01
Amikacin ≥ 3 days	13 (30)	30 (10)	0.0002
Any non-antipseudomonal antibiotic	40 (91)	206 (68)	0.002
Any non-antipseudomonal antibiotic ≥ 3 days	31 (70)	137 (45)	0.002
Colonization pressure, mean ± SD	0.17 ± 0.14	0.15 ± 0.14	0.3
Enteral nutrition	35 (80)	86 (28)	<0.0001
Parenteral nutrition	11 (25)	39 (13)	0.03
Nasogastric tube	43 (98)	207 (69)	0.0001
Tracheostomy	12 (27)	27 (10) (9)	0.0003
Mechanical ventilation	41 (93)	189 (63)	0.0001
Renal replacement	5 (11)	17 (6)	0.17
Central venous catheter	27 (61)	130 (43)	0.02
Corticosteroids	32 (73)	161 (53)	0.015

If not otherwise stated figures express number of patients (%). Multivariate analysis selected the following variables: enteral nutrition (OR 11.4, 95% CI 4.9–26.4), prior administration of quinolones (OR 0.27, 95% CI 0.1–0.7), prior administration of antipseudomonal cephalosporins (OR 0.27, 95% CI 0.08–0.9), use of piperacillin-tazobactam for ≥ 3 days (OR 2.6, 95% CI 1.09–6.27) and administration of amikacin for ≥ 3 days (OR 2.6, 95% CI 1.04–6.7)

APACHE acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment, *IQ* interquartile range, *COPD* chronic obstructive pulmonary disease

antibiotic refers to the conversion of a genotypically defined strain from susceptible to non-susceptible. Therefore, patients who fulfilled the "emergence of resistance" definition were the subset of patients that belonged to the "acquisition of resistance" group whose previously susceptible strain developed resistance. Time at risk means the time elapsed from ICU admission to the isolation of *P. aeruginosa* for patients acquiring the organisms or the length of ICU stay for those not acquiring the pathogen. Antibiotic exposure means at least 24 h of treatment. Colonization pressure with *P. aeruginosa* was estimated as previously described [35].

Statistical analysis

Clinical variables and exposures were compared between patients who acquired *P. aeruginosa* and those who did not; between patients who eventually carried a strain resistant to a given antipseudomonal agent that was either susceptible or not present on admission and those who did not; and, among patients colonized with a strain of *P. aeruginosa* susceptible to a given antipseudomonal agent, the same variables were compared between those in whom resistance to that antibiotic emerged and those in whom did not. For patients who reached the first two

outcomes, evaluable exposures had to be present any time before detection of the defining event. For the third outcome, evaluable exposures had to be present from detection of the susceptible strain to isolation of its resistant counterpart.

Proportions were compared by using the χ^2 or Fisher's exact test. Continuous variables were compared by using the *t* test or Mann–Whitney test. A stepwise logistic regression procedure was used to select the best predictors of *P. aeruginosa* acquisition and acquisition of resistance to cephalosporins and quinolones. Only those variables with a *P* value ≤ 0.1 in the univariate analysis were entered into the logistic models. No attempt was made to perform multivariate analysis when the number of responses defining the outcome was less than 20.

Results

A total of 346 patients was evaluated, of whom 44 (13%) acquired 52 strains of *P. aeruginosa* during their ICU stay (37 patients acquired a sole strain, six acquired two and one acquired three). Twenty-eight (8%) additional patients were colonized on admission. Acquired isolates belonged to 17 unique genotypes, of which 9 were recovered from more than one patient. As a matter of fact, two unique strains colonized 50% of patients (one was acquired by 13, the other by eight and one patient acquired both). Out of the 52 isolates, 21 (40%) in 19 patients were acquired via cross-transmission and the remaining were of endogenous or unknown origin. Sites of primary detection included the rectum in 20 patients (45%), the nares or pharynx in 11 (25%), the lower respiratory tract in 3 (7%), more than one of these places in 9 (20%) and other sites (skin) in one (2%). The median time to *P. aeruginosa* acquisition was 6 days (IQ range 4.25–11.5, range 3–37).

Two-hundred and forty-three patients (70%) received an antipseudomonal agent during their ICU stay; 87 (25%) were exposed to cephalosporins (88% to cefepime), 97 (28%) to carbapenems (70% to imipenem), 81 (23%) to piperacillin-tazobactam, 126 (36%) to quinolones (89% to ciprofloxacin) and 83 (24%) to amikacin. Ninety-one patients received only one antipseudomonal agent (13 a cephalosporin, 20 a carbapenem, 26 piperacillin-tazobactam, 38 a quinolone and 4 amikacin), 100 received a single antipseudomonal β-lactam and either a quinolone ($n = 51$), amikacin ($n = 34$) or both ($n = 15$), 22 received more than one β-lactam, 8 received more than one β-lactam and amikacin, 21 received more than one β-lactam and both a quinolone and amikacin, and a patient received a quinolone and amikacin. Quinolones ($n = 88$, 70%) and amikacin ($n = 79$, 95%) were preferentially used in combination with other antipseudomonal agents. The median daily dosages were 4 g for cephalosporins, 3 g for carbapenems, 12 g for piperacillin-tazobactam, 800 mg for ciprofloxacin, 500 mg for levofloxacin, and 1 g for amikacin (given always as a single daily dose). Almost all patients ($n = 331$, 99%) received some antibiotic for at least 24 h during their ICU stay. Median (interquartile range) days of exposure were 4 (2–7) for cephalosporins, 6 for carbapenems (2–11.5), 5 (2–8) for piperacillin-tazobactam, 4 for quinolones (2–8), 6 for amikacin (2–9) and 5 (1.75–8) for other antimicrobial agents.

Risk factors for the acquisition of *P. aeruginosa*

The relation between patient characteristics or exposures and the acquisition of *P. aeruginosa* is shown in Table 1. In regards to the previous use of antibiotics, there was a significant positive association with piperacillin-tazobactam, amikacin and non-antipseudomonal agents, whereas the administration of quinolones and antipseudomonal cephalosporins was protective. Multivariate analysis selected the following variables as independently associated with the acquisition of *P. aeruginosa*: enteral nutrition (OR 11.4, 95% CI 4.9–26.4), prior administration of quinolones (OR 0.27, 95% CI 0.1–0.7), prior administration of antipseudomonal cephalosporins (OR 0.27, 95% CI 0.08–0.9), use of piperacillin-tazobactam during three days or longer (OR 2.6, 95% CI 1.09–6.27) and administration of amikacin during three days or longer (OR 2.6, 95% CI 1.04–6.7).

Relation between acquisition of resistance to antipseudomonal agents and prior exposure to the same or a different agent

The number of patients in whom a *P. aeruginosa* isolate resistant to the different antipseudomonal agents was detected during ICU stay, the resistance status of these strains when first isolated and the number of cases of cross-transmission are shown in Table 2. In most cases, resistance emerged from previous susceptible strains. Univariate analysis assessing the relationship between acquisition of resistance to the different antipseudomonal agents and prior exposure to each agent is shown in Table 3. Neither the use of antipseudomonal cephalosporins nor quinolones was associated with the acquisition of resistance. Conversely, administration of carbapenems was associated with acquisition of resistance to themselves, piperacillin-tazobactam and amikacin; use of piperacillin-tazobactam was associated with resistance to antipseudomonal cephalosporins, and use of amikacin was associated with the acquisition of resistance to itself,

Table 2 Number of patients in whom a *P. aeruginosa* strain resistant to the different antipseudomonal antibiotics was isolated during ICU stay, resistance status of the strains when first detected and number of cases of cross-transmission (in brackets)

Antibiotic	No. of patients	On admission as susceptible	Acquired in ICU as susceptible	Acquired in ICU as resistant
Cephalosporins	26	5 (19%)	11 (42%) [5]	10 (38%) [6]
Carbapenems	13	5 (38%)	6 (46%) [5]	2 (15%) [0]
Piperacillin-tazobactam	5	0	4 (80%) [2]	1 (20%) [0]
Quinolones	27	6 (22%)	12 (44%) [8]	9 (33%) [4]
Amikacin	9	1 (11)	7 (78%) [4]	1 (11%) [1]

On admission, eight patients carried an isolate resistant to at least one antipseudomonal agent: one patient to cephalosporins, two to cephalosporins plus quinolones, one to cephalosporins plus piperacillin-tazobactam plus quinolones, one to cephalosporins plus carbapenems plus quinolones, one to carbapenems and two to quinolones

Table 3 Relation between acquisition of resistance to antipseudomonal antibiotics and prior exposure to the same or a different agent

Acquisition of resistance to	Number of patients (%)	Number (%) of patients previously exposed to									
		Ceftazidime/ cefepime	*P*	Carbapenem	*P*	Piperacillin-tazobactam	*P*	Quinolones		Amikacin	*P*
Cephalosporins[a,b]											
Yes	26	4 (15)		9 (35)		12 (46)		9 (35)		9 (35)	
No	320	78 (24)	0.3	83 (26)	0.3	69 (22)	0.004	114 (36)	0.9	69 (22)	0.1
Carbapenems[c]											
Yes	13	4 (31)		8 (62)		3 (23)		5 (39)		9 (69)	
No	333	83 (25)	0.6	84 (25)	0.007	78 (23)	1	118 (35)	0.7	71 (21)	0.0004
Piperacillin-tazobactam[d]											
Yes	5	0		4 (80)		2 (40)		2 (40)		3 (60)	
No	341	87 (26)	0.3	93 (27)	0.02	78 (23)	0.3	122 (36)	1	79 (23)	0.08
Quinolones[e,f]											
Yes	27	6 (22)		8 (30)		6 (22)		7 (26)		11 (41)	
No	319	75 (24)	1	82 (26)	0.6	71 (22)	1	113 (35)	0.4	68 (21)	0.03
Amikacin[g]											
Yes	9	2 (22)		6 (67)		2 (22)		3 (33)		7 (78)	
No	337	83 (25)	1	90 (27)	0.01	79 (23)	1	121 (36)	1	75 (22)	0.0008

Figures express number of patients (%). [a] Other variables with an univariate significant association were: shock (11, 42 vs. 74, 23%, $P = 0.04$), central venous catheter (20, 77 vs. 137, 43%, $P = 0.001$), enteral nutrition (19, 73 vs. 113, 35%, $P = 0.0002$), nasogastric tube (26, 100 vs. 232, 73%, $P = 0.0006$), tracheostomy (9, 35 vs. 39, 12%, $P = 0.005$) and mechanical ventilation (25, 96 vs. 213, 67%, $P = 0.0007$). [b]Multivariate analysis selected enteral nutrition (OR 4.52, 95% CI 1.82–11.2) and exposure to piperacillin-tazobactam (OR 2.6, 95% CI 1.12–6) [c]Other variables with an univariate significant association were: shock (7, 54 vs. 78, 23%, $P = 0.02$), central venous catheter (11, 85 vs. 146, 44%, $P = 0.004$), enteral nutrition (11, 85 vs. 121, 36%, $P = 0.0007$) and nasogastric tube (13, 100 vs. 244, 73%, $P = 0.025$). [d] Other variables with an univariate significant association were: enteral nutrition (5, 100 vs. 129, 38%, $P = 0.008$) and tracheostomy (4, 80 vs. 49, 14%, $P = 0.002$). [e] Other variables with an univariate significant association were: central venous catheter (18, 67 vs. 139, 44%, $P = 0.03$), enteral nutrition (23, 85 vs. 110, 35%, $P < 0.0001$), nasogastric tube (26, 96 vs. 230, 72%, $P = 0.005$), mechanical ventilation (26, 96 vs. 211, 66%, $P = 0.0004$) and corticosteroids use (22, 82 vs. 171, 54%, $P = 0.005$). [f]Multivariate analysis selected only enteral nutrition (OR 10.9, 95% CI 3.67–32.5). [g]Other variables with an univariate significant association were: enteral nutrition (8, 89 vs. 126, 37%, $P = 0.003$) and tracheostomy (4, 44 vs. 48, 14%, $P = 0.03$)

carbapenems and quinolones. Multivariate analysis selected enteral nutrition as the only risk factor independently associated with acquisition of quinolone resistance (OR 10.9, 95% CI 3.67–32.5), and enteral nutrition (OR 4.52, 95% CI 1.82–11.2) and exposure to piperacillin-tazobactam (OR 2.6, 95% CI 1.12–6) as the main predictors of cephalosporin resistance acquisition. After adjusting for enteral nutrition and exposure to piperacillin-tazobactam, admission during the mixing periods showed a trend towards association with the acquisition of cephalosporin resistance (OR 2.33, 95% CI 0.95–5.68).

Emergence of resistance after exposure and non-exposure of susceptible isolates to the different antipseudomonal agents

The risk of resistance emergence in patients previously colonized with susceptible strains of *P. aeruginosa* after exposure and non-exposure to the corresponding classes of antipseudomonal antibiotics is shown in Table 4. Exposure to any antibiotic, administered alone or in combination, was endowed with some risk for the emergence of resistance and, in this regard, combination

Table 4 Emergence of resistance in patients exposed (any exposure or for ≥ 3 days) and non-exposed to the specified antibiotic after documented colonization with the same susceptible pulsotype of *P. aeruginosa*

Antibiotic	Any exposure Patients in whom resistance emerged/patients exposed (%)	Exposure ≥ 3 days Patients in whom resistance emerged/patients exposed (%)	Non-exposure Patients in whom resistance emerged/patients not exposed (%)	P^a
Cephalosporins	3/12 (25)	3/7 (43)	13/44 (30)	1
Alone	0/2	0		
Plus amikacin	1/3 (33)	1/3 (33)		
Plus quinolone	2/7 (29)	2/4 (50)		
Any combination	3/10 (30)	3/7 (43)		
Carbapenems	7/18 (39)	7/15 (47)	4/50 (8)	0.005
Alone	5/12 (42)	5/9 (56)		
Plus amikacin	2/4 (50)	2/4 (50)		
Plus quinolone	0/2	0/2		
Any combination	2/6 (33)	2/6 (33)		
Piperacillin-tazobactam[b]	2/23 (9)	2/18 (11)	2/47 (4)	0.6
Alone	1/17 (6)	1/12 (8)		
Plus amikacin	1/6 (17)	1/5 (20)		
Plus quinolone	0/1	0/1		
Any combination	1/7 (14)	1/6 (17)		
Quinolones	5/18 (28)	5/14 (42)	13/39 (33)	0.7
Alone	2/6 (33)	2/5 (40)		
Any combination	3/12 (25)	3/9 (33)		
Amikacin	5/15 (33)	5/12 (42)	3/55 (5)	0.009
Alone	2/2 (100)	2/2 (100)		
Any combination	3/13 (23)	3/10 (33)		

Figures express number of patients (%)

[a] Proportions of the first and third columns are compared

[b] Variables significantly associated in univariate analysis were: tracheostomy [resistance emerged in 4 of 28 (14%) patients with tracheostomy versus 0 of 46 without it, $P = 0.02$] and carbapenem use [resistance emerged in 3 of 18 (17%) patients exposed vs. 1 of 52 (2%) non-exposed, $P = 0.049$]. There were no other variables significantly associated with the emergence of resistance to cephalosporins, carbapenems, quinolones or amikacin

therapy was apparently devoid of any preventive activity. Emergence of resistance was somewhat lower for piperacillin-tazobactam than for other antibiotics, although only the difference with carbapenems reached statistical significance (9 vs. 39%, $P = 0.03$). Five out of the eight courses of combination therapy which failed to prevent resistance were associated with the loss of susceptibility to the two components of the association. Resistance always arose after exposure to a given antibiotic for at least 3 days.

Emergence of resistance to quinolones and cephalosporins occurred at similar frequencies in patients exposed and not exposed to these agents, whereas carbapenem and amikacin resistance was clearly associated with the administration of these antibiotics.

Discussion

The present study provides some unique data concerning the putative role of antipseudomonal agents on their ability to promote *P. aeruginosa* colonization, acquisition of resistant strains and emergence of resistance in critically ill patients. In regards to the first outcome, our data suggest that quinolones and antipseudomonal cephalosporins may actually prevent the acquisition of *P. aeruginosa*, whereas piperacillin-tazobactam and amikacin may enhance it. With respect to the acquisition of resistance, we found that quinolones and cephalosporins were rather neutral, whereas all the other agents were associated with the acquisition of resistance to themselves or other antibiotics. Finally, emergence of resistance to any of the antipseudomonal agents certainly occurred after administering the corresponding antibiotic to patients previously colonized by susceptible strains, but for quinolones and cephalosporins this was not more frequent in patients who took the antimicrobial than in those who did not. Finally, the present data also suggest that emergence of resistance never arose to detectable levels before three days of continuous therapy and that combination treatment was not useful for prevention.

The role of systemic agents with antipseudomonal activity on the acquisition of *P. aeruginosa* in ICU patients has not been adequately explored. Our finding that some agents may have a significant preventive effect is not entirely unexpected. The impressive record of quinolones in preventing infections due to gram-negative bacilli, even in the surge of the current wave of resistance among common enteric bacilli and non-fermenters [36], is probably still due to their ability to decolonize mucosal surfaces and deter the acquisition of

susceptible exogenous flora. It is more difficult to explain the discordant effect of antipseudomonal cephalosporins on one hand and piperacillin-tazobactam or amikacin on the other.

When acquisition of resistance to the different antipseudomonal agents is considered, the present data clearly diverge from those of other studies, particularly in regards to the role of quinolones. At least four studies have linked prior administration of quinolones with the acquisition of quinolone-resistant *P. aeruginosa* in either the gastrointestinal tract [3], any clinical sample [14, 19] or bloodstream isolates [15]. In addition, other investigations have shown an association between quinolone usage and resistance to piperacillin-tazobactam [31], imipenem [21, 25] and multidrug resistance [24, 29]. Although studies differ in design, and some have compared patients with resistant versus susceptible strains [14, 15, 19] (a strategy prone to magnify the risk associated with quinolone usage), it is difficult to ascertain the true reason for the discrepancy with the present data. It may rely, in part, on the predominant use of ciprofloxacin in our patients, a quinolone that, contrarily to levofloxacin, in some studies has not been associated with the acquisition of quinolone-resistant *P. aeruginosa* [13, 20, 37]. On the other hand, our sampling procedure, being more frequent and thorough than that of other studies, not only increased the chances of detecting *P. aeruginosa* but also provided a more accurate time estimation of the evaluated outcomes and the actual moment of antibiotic exposure in relation to them. The lack of association of quinolone usage with acquisition of resistance to carbapenems, piperacillin-tazobactam and amikacin may have been due, in part, to the small sample size. It must be remembered, however, that some studies have found quinolones to be protective against cephalosporin resistance in gram-negative bacilli, including *P. aeruginosa* [38].

The strategy of antibiotic use (mixing or cycling) was not influential in the acquisition of *P. aeruginosa*, but during mixing there was a non-significant trend towards an increased risk of resistance acquisition to cephalosporins. This is consistent with our previous observation that cycling was better than mixing in regards to the acquisition of *P. aeruginosa* resistant to selected β-lactams, particularly cefepime [32]. In the present study, enteral nutrition was the sole factor apparently associated with the acquisition of strains with a quinolone-resistant phenotype and was also an important predictor of the acquisition of strains resistant to cephalosporins and other antipseudomonal agents. Enteral feeding has been previously reported as an independent risk factor for multidrug resistance [24]. Although we do not have a plausible explanation for this finding, we think that enteral feeding could be a marker for some non-assessed variable involved in transmission.

Other associations found in the present study have been previously observed, such as the involvement of prior administration of imipenem and amikacin in imipenem resistance [9, 11, 18, 21], previous use of imipenem in piperacillin-tazobactam resistance [12], and prior exposure to piperacillin in ceftazidime resistance [26]. Our data also suggest that the use of carbapenems may predispose to the acquisition of amikacin resistance, and amikacin to quinolone resistance. Many, albeit not all of these associations can be explained by the selection of mutants overexpressing different multidrug efflux systems of the resistance-nodulation-division family [39]. However, studies aimed to correlate the specific mechanisms of resistance with prior antibiotic exposure are necessary to clarify this issue.

In the present study, emergence of resistance was observed after exposure to any of the antipseudomonal agents at frequencies within the range previously described in the literature. However, some intriguing differences were again noted between quinolones and cephalosporins on one hand and the remaining agents on the other. For all antibiotics we clearly documented that emergence of resistance occurred without exposure to the corresponding agent, which, in the case of quinolones and cephalosporins, was as common as in patients actually exposed. Although the number of treated patients was low, combination therapy did not seem to prevent the emergence of resistance, but the value of this strategy has never been consistently demonstrated in the clinical scenario [40].

Patients could end up carrying a strain resistant to a given antipseudomonal agent by acquisition of an already resistant isolate from other patients or an unknown source or by selection of resistance on a previously susceptible strain. The number of patients who acquired resistance due to cross-transmission of a resistant stain ranged from 0 for carbapenems and piperacillin-tazobactam to 6 (23%) for cephalosporins (Table 2). The respective roles of patient-to-patient transmission of a resistant clone and selection of resistance may depend on the proportion of other patients being colonized by strains resistant to the antibiotic considered (colonization pressure). In this study, however, colonization pressure was not associated with the acquisition of *P. aeruginosa* or of resistance. This may have been due to the relative few outcomes observed and to the low values of colonization pressure, which exceptionally were $\geq$0.5 [35].

The present study has several caveats, the most obvious being the relatively low number of observed outcomes of interest, hence many of the associations found should be confirmed in larger studies. On the other hand, the results may have been influenced by local epidemiological variables not applicable to other settings. Some of these potential confounders may include a relative high rate of horizontal transmission, almost universal exposure to antibiotics, preferential use of ciprofloxacin over levofloxacin and the frequent use of quinolones in combination regimens. In addition, we dealt with colonizing strains prospectively searched by an active

sampling program, which could affect the clinical relevance of our observations.

In conclusion, the influence of antipseudomonal antibiotics on the risk of colonization by *P. aeruginosa,* acquisition of a resistant phenotype or the emergence of resistance of this pathogen depends on the particular antimicrobial agent. At least in some critical care settings, quinolones and antipseudomonal cephalosporins may actually prevent the acquisition of *P. aeruginosa* and may have a negligible effect on the acquisition and emergence of resistance.

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