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Effects of Carbapenem Exposure on the Risk for Digestive Tract Carriage of Intensive Care Unit-Endemic Carbapenem-Resistant *Pseudomonas aeruginosa* Strains in Critically Ill Patients▽

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To determine the epidemiology and risk factors for carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA) digestive tract colonization, weekly rectal and pharyngeal swabs were obtained in two serial incidence surveys (266 patients). Forty-two (16%) patients were CR-PA colonized (12 [29%] on admission and 30 [71%] in intensive care units). Pulsed-field gel electrophoresis showed extensive clonal diversity, although one specific clone (type B) was isolated from 11 patients. The presence of similar genotypes of CR-PA colonizing 30% of the CR-PA-colonized patients suggests the occurrence of cross-colonization; in addition, 10 pairs of carbapenem-susceptible *P. aeruginosa* (CS-PA) and subsequent CR-PA strains isolated from the same patients were found to be clonally identical and were considered to have been endogenously acquired (33%). All endogenously acquired CR-PA strains were isolated after exposure to a carbapenem, and 80% showed a phenotype of imipenem resistance (IR pattern) alone, while 67% of the CR-PA strains acquired by cross-transmission exhibited a multiresistant (MR) phenotype, with previous carbapenem exposure in 44%. Logistic regression analysis identified severity of acute illness (odds ratio [OR], 1.0; 95% confidence interval [CI], 1.0 to 1.1), prior carbapenem use (OR, 7.8; 95% CI, 1.7 to 35.3), and prior use of fluoroquinolones (OR, 11.0; 95% CI, 1.7 to 67.9) as independent risk factors for CR-PA digestive tract colonization. Overall, the local epidemiology of CR-PA digestive tract colonization was characterized by polyclonal endemicity with phenotype patterns of IR and MR divided evenly between patients. Restricting the use of particular agents, such as carbapenems and fluoroquinolones, should be considered advisable to minimize the problem of this antibiotic resistance. However, the possible risk for development of collateral unexpected bacterial resistance patterns should be accurately monitored.

Pseudomonas aeruginosa is recognized as one of the most common hospital-acquired pathogens worldwide, and it has become endemic in many intensive care units (ICUs). Epidemiologic analyses in the ICU setting have usually considered these infections following a secondary endogenous colonization of patients (6, 10), but some aspects of the source and mode of transmission of the microorganism remain controversial (3, 6). In fact, recent studies have shown moist sites to be the primary source of endemic spread of *P. aeruginosa* colonization, being found in at least a third of ICU patients (22, 26). In addition, the environment and colonized patients are continuous exogenous sources from which other patients can be colonized via the transitorily colonized hands of health care workers (3, 4, 9).

The isolation of carbapenem-resistant *P. aeruginosa* (CR-PA) strains because of the loss of a specific OprD porin following exposure of the microorganism to these antibiotics was already reported 2 decades ago (11). However, more recently multidrug resistance has progressively emerged in CR-PA isolates as a consequence of the development of other mechanisms of resistance such as efflux pumps or of the concomitant appearance of several mechanisms of resistance to antibiotics of different kinds (15).

While the general epidemiological characteristics of CR-PA infections appear to resemble those of nosocomial *P. aeruginosa*, there have been few epidemiological studies of ICUs with high rates of multidrug CR-PA (17). Determining the relevance of endogenous colonization versus cross-transmission and the influence of carbapenems and other families of antibiotics is essential to the design of targeted and efficacious strategies for infection control.

The high prevalence of CR-PA isolates in our ICUs, where they are apparently endemic, prompted us to conduct a prospective surveillance study in order to characterize the local epidemiology and to identify risk factors for CR-PA carriage.

MATERIALS AND METHODS

This study was performed in two 12-bed medical-surgical ICUs at the Hospital Universitari de Bellvitge, a 900-bed hospital. The units have different medical and nursing staffs, with one nurse for every two patients, except in the case of patients who have undergone organ transplantation, who each have one nurse. The paramedic care providers (physiotherapists, radiographic personnel, nutrition support team) have patient contacts in both ICUs. All rooms in the ICUs are single rooms. There have been no outbreaks of multiply resistant microorganisms in the last 3 years.

Study design. We carried out an active surveillance program with ICU patients over two different 2-month periods (May and June of 2003 and 2004). Weekly rectal and oropharyngeal swab samples were obtained immediately on admission to detect digestive tract carriage of CR-PA between ICU admission and discharge. To study patients at risk for digestive tract carriage of CR-PA during their ICU stay, all patients admitted to the unit for more than 48 h were included.

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Demographic characteristics and severity of acute illness on ICU admission were recorded by using the simplified acute physiologic score (SAPS) (14). We also assessed the devices in place, i.e., intravascular catheters, urinary catheters, and endotracheal tubes, and prior surgery. Antibiotic therapy was determined as the number of days of therapy with different groups of antibiotics for each patient since hospital admission until CR-PA colonization. The groups of antibiotics analyzed were penicillins (penicillin G, ampicillin, amoxicillin-clavulanic acid, cloxacillin, and piperacillin-tazobactam), cephalosporins (all cephalosporins and aztreonam), aminoglycosides, glycopeptides, fluoroquinolones, and carbapenems.

Antibiotic pressure was determined according to the consumption of different groups of antibiotics per month. The period studied included the 2-months previous to the surveillance program (March and April of 2003 and 2004) and antibiotic consumption during the study period (May and June of 2003 and 2004). As recommended by the Nordic Council on medicines (20), consumption was expressed as defined daily doses (DDD). During the study period, the use of antibiotics in the ICUs was not restricted, in accordance with hospital policy. However, scheduled antimicrobial cycling was used in these wards during the second period.

Microbiological surveillance and genotyping. Susceptibility studies. MICs were determined with the MicroScan automated microdilution system (Dade International, West Sacramento, CA). The antibiotics tested were piperacillin, ticarcillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin, and amikacin. The criteria of the National Committee for Clinical Laboratory Standards (19) were used to define susceptibility or resistance to these antimicrobial agents. Intermediate isolates were considered resistant.

CR-PA colonization detection. Rectal and oropharyngeal swabs were inoculated onto MacConkey agar plates. The plates were incubated at 37°C for 48 h. Gram-negative, oxidase-positive bacilli growing on MacConkey agar were identified as *P. aeruginosa* by conventional biochemical tests.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNAs from all CR-PA and earlier CS-PA digestive tract samples from ICU patients were prepared for PFGE analysis as previously described (23) and then digested with XbaI. DNA fragments were separated with a CHEF DR III apparatus (Bio-Rad Laboratories, Hercules, CA). Electrophoresis was run at 6 V/cm and 14°C for 23 h with pulses ranging from 5 to 25 s. DNA restriction patterns generated by PFGE were interpreted according to the guidelines proposed by Tenover et al. (24).

Definitions. Carriage was defined as at least one positive culture obtained from an oropharyngeal or rectal swab. CR-PA carriage on admission was defined as a positive swab culture within 48 h of ICU admission. Colonizations occurring more than 48 h after ICU admission were defined as ICU acquired.

We used the following criteria to provide an epidemiological classification of the colonization routes in the digestive tract. (i) Endogenous colonization was considered if the finding of CR-PA was preceded by isolation of carbapenem-susceptible *P. aeruginosa* (CS-PA) in the same patient and if the strains showed identical clonal patterns. (ii) Exogenous colonization or cross-transmission was considered if CR-PA isolates had a PFGE pattern similar to that of isolates from at least one other patient present in either ICU. (iii) Indeterminate colonization was considered if the patient did not have endogenous colonization according to the above criteria and if the colonization involved a CR-PA strain not identified in other patients by PFGE.

In addition, two patterns of carbapenem resistance were differentiated in CR-PA isolates. (i) An imipenem resistance (IR) pattern was considered when strains showed resistance to imipenem along with an increase in the MICs of meropenem but within the susceptibility range. (ii) A multiresistance (MR) pattern was considered if strains showed resistance to imipenem and meropenem and concomitantly to at least two other families of antibiotics.

Statistical analysis. Contingency tables were analyzed by two-tailed chi-square test. Continuous variables were compared by the *t* test. Univariate analysis was performed to determine the significance of risk factors for CR-PA digestive tract carriage acquisition. $P < 0.05$ was considered statistically significant. A multivariate analysis was performed to assess the independence of statistically significant variables in the univariate analysis by an unconditional stepwise logistic regression model. The probability of carriage of CR-PA in the digestive tract was calculated by using the Kaplan-Meier estimate. Statistical analysis was performed with the SPSS/PC version 11.0 statistical package.

RESULTS

Study population. During the two study periods (May and June of 2003 and 2004), 278 patients were hospitalized in the ICUs. Among these 278 patients, 12 patients with ICU stays of less than 48 h were excluded. The remaining 266 patients were included in the present study, 42 of whom (16%) were CR-PA colonized, 12 (29%) at ICU admission and the remaining 30 (71%) during their ICU stay. Among the 12 patients colonized at ICU admission, none had been hospitalized previously in an ICU and their median prior hospital stay was 40 days (range, 7 to 117 days).

The remaining 254 patients were included in the study cohort, 119 patients from May and June of 2003 and 135 patients from May and June of 2004. The epidemiological characteristics in the two periods were comparable, although patients in the first period had received more prior antibiotics than patients in the second period (87 [84%] versus 84 [62%]; $P < 0.01$), specifically, more carbapenems (43 [36%] versus 21 [15.5%]; $P < 0.01$).

Risk factors for CR-PA carriage. Thirty digestive tract CR-PA carriers were colonized during the two study periods, 17 (14%) out of 119 patients during the first period and 13 (10%) of 135 patients during the second period. The probabilities of CR-PA carriage in the digestive tract were 10 and 8%, respectively, 10 days ($P = 0.8$) after ICU admission (Fig. 1).

Aminoglycosides, glycopeptides, cephalosporins, and penicillins were used regularly during the two study periods. However, carbapenem use decreased from 174 DDD/1,000 patient days (March to June 2003) to 122 DDD/1,000 patient days (March to June 2004). There was a concomitant increase in fluoroquinolone use, from 151 DDD/1,000 patient days in 2003 to 213 DDD/1,000 patient days in 2004.

The variables associated with CR-PA carriage are listed in Table 1. CR-PA carriers were more likely to have received antibiotics than non-CR-PA carriers (93 versus 64%). In addition, these CR-PA carriers were more likely to have been exposed to carbapenems (63 versus 20%) and fluoroquinolones (23 versus 7%). A logistic regression model with CR-PA digestive tract colonization as the dependent variable identified the severity of acute illness according to SAPS (odds ratio [OR], 1.0; 95% confidence interval [CI], 1.0 to 1.1), prior carbapenem use (OR, 7.8; 95% CI, 1.7 to 35.3), and prior use of fluoroquinolones (OR, 11.0; 95% CI, 1.7 to 67.9) as independent risk factors.

Microbiological and genotypic analysis. Table 2 presents the breakpoint resistance and the percent resistance to antipseudomonal antibiotics of our CR-PA strains. Twenty-five (59.5%) CR-PA strains showed an MR pattern; the remaining 17 CR-PA isolates (40.5%) had a uniform phenotype pattern with resistance to imipenem alone (IR pattern). All isolates were susceptible to colistin.

Twenty-three genotypes were found in the 42 CR-PA isolates. The PFGE profiles of the 25 isolates with an MR phenotype showed 11 different clones, one of which, B, was isolated from 11 patients.

Acquisition of CR-PA carriage. Among the 30 colonized patients with ICU acquisition, 15 genotypes were found in a single patient only, whereas 1 genotype, B, was isolated from 5 patients, 2 other genotypes (J and L) were isolated from 4 and 2 patients, respectively, and isolates were nontypeable in four cases (Table 3).

Fifteen patients had previous CS-PA digestive tract coloni-

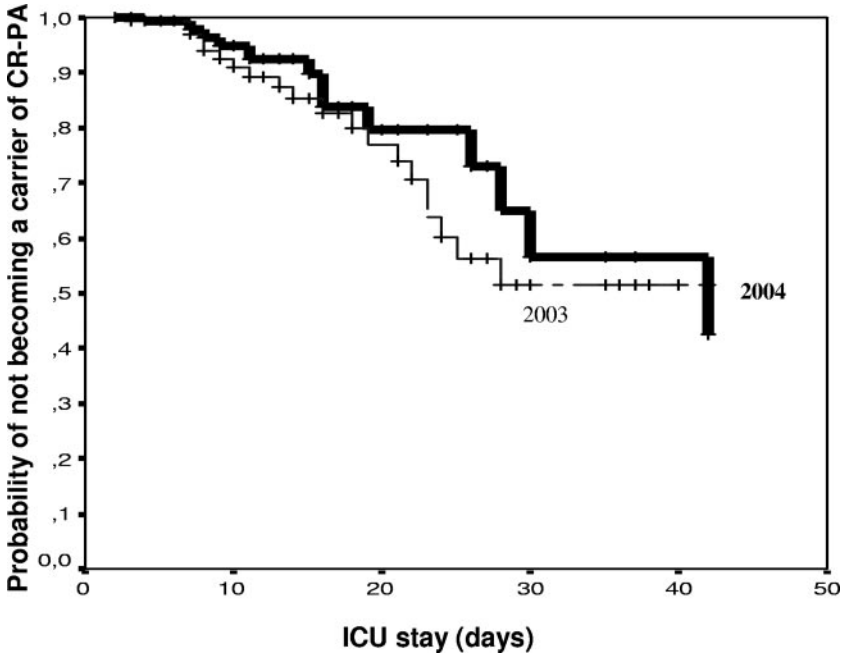

FIG. 1. Probability of remaining free of digestive tract CR-PA colonization. Thin line, first period; thick line, second period.

TABLE 1. Univariate analysis of risk factors for digestive tract CR-PA colonization of ICU patients

Characteristic	Patients CR-PA colonized (n = 30)	Patients not CR-PA colonized (n = 224)	P value
Mean age (yr) ± SD	59.8 ± 15.0	57.1 ± 15.7	0.3
No. (%) of males/females	18 (60)/12 (40)	145 (65)/79 (35)	0.6
No. (%) with immunosuppression	3 (10)	36 (16)	0.5
No. (%) with neutropenia	1 (3)	7 (3)	1.0
No. (%) with malignancy or AIDS	2 (7)	31 (14)	0.3
No. (%) tested in 2003	17 (57)	102 (45.5)	
No. (%) tested in 2004	13 (43)	122 (54.5)	0.2
No. (%) with prior CS-PA	15 (50)	75 (33)	0.01
No. (%) with antibiotic exposure	28 (93)	143 (64)	<0.01
No. (%) with carbapenem exposure	19 (63)	45 (20)	<0.01
No. (%) with fluoroquinolone exposure	7 (23)	15 (7)	0.01
No. (%) with vancomycin exposure	4 (13)	27 (12)	0.7
No. (%) with cephalosporin exposure	1 (3)	16 (7)	0.7
No. (%) with amikacin exposure	8 (27)	32 (14)	0.1
No. (%) with piperacillin-tazobactam exposure	3 (10)	17 (7.5)	0.7
No. (%) with previous surgery	13 (43)	59 (26)	0.05
No. (%) with mechanical ventilation	26 (87)	139 (62)	<0.01
No. (%) with a urinary catheter	27 (90)	194 (87)	0.7
No. (%) with a venous catheter	28 (93)	188 (84)	0.06
No. (%) with total parenteral nutrition	13 (39)	33 (15)	<0.001
Mean SAPS[a] ± SD	40.6 ± 10.4	28.0 ± 14.8	<0.001
Mean ICU stay (days) ± SD	16.3 ± 9.0	8.6 ± 8.0	<0.001

[a] SAPS at time of ICU admission.

zation, and in these cases, pairs of CS-PA and CR-PA strains were studied by PFGE. In 10 patients (33%), a clonal identity was demonstrated and CR-PA colonization was considered to be endogenous; in the remaining 5 patients, the strains were noncoincident. In these patients with endogenous acquisition, CR-PA strains were isolated after carbapenem exposure and the CR-PA phenotype showed an IR pattern in eight patients (80%) and an MR pattern in only two patients (20%). In nine patients (30%), CR-PA acquisition was considered to be exogenous and six (67%) of these CR-PA strains exhibited an MR phenotype. In the remaining 11 (37%) patients, no colonization route could be demonstrated; in 7, acquisition was considered indeterminate and 4 patients were not evaluated since their CR-PA isolates were nontypeable. The indeterminate-acquisition patients showed CR-PA strains with an MR phenotype in five cases. Among the 26 evaluable patients, the acquisition route was distributed similarly in the two periods (Table 3).

Among the 12 CR-PA digestive tract colonizations before

TABLE 2. Activities of antibiotics against CR-PA isolates

Antibiotic	MIC (μg/ml)[a]	No. of isolates resistant/total[a] (%)
Piperacillin	>64	9/42 (21)
Ticarcillin	>64	12/42 (28.5)
Ceftazidime	>8	12/42 (28.5)
Aztreonam	>8	14/42 (33)
Gentamicin	>4	26/42 (62)
Tobramycin	>4	23/42 (55)
Amikacin	>16	8/42 (19)
Ciprofloxacin	≥2	25/42 (59.5)
Imipenem	≥8	42/42 (100)
Meropenem	≥8	25/42 (59.5)

[a] The number and percentage of resistant isolates were defined according to NCCLS nonsusceptibility breakpoints and therefore include both the intermediate and resistant categories.

TABLE 3. Routes of CR-PA colonization of ICU patients

Patient	Yr of study	CS-PA clone	CR-PA clone	CR-PA phenotype (resistance pattern)	Previous carbapenem or fluoroquinolone therapy	Type of acquisition
1	2003	P	P	IR	Imipenem	Endogenous
2	2003	J	J	IR	Imipenem	Endogenous
3	2003	L	L	MR	Imipenem	Endogenous
4	2003	E	E	IR	Imipenem, ciprofloxacin	Endogenous
5	2003	K	K	MR	Imipenem, ciprofloxacin	Endogenous
6	2003	O	B	MR	Imipenem	Exogenous
7	2003	Not isolated	B	MR	Meropenem	Exogenous
8	2003	Not isolated	J	IR	Imipenem	Exogenous
9	2003	Not isolated	J	IR	Unknown	Exogenous
10	2003	C	H	MR	Imipenem	Indeterminate
11	2003	Not isolated	N	MR	Imipenem	Indeterminate
12	2003	Not isolated	D	IR	Imipenem	Indeterminate
13	2003	Not isolated	O	IR	Imipenem	Indeterminate
14	2003	Not isolated	Nontypeable	MR	None, ciprofloxacin	Not evaluated
15	2003	Not isolated	Nontypeable	MR	None	Not evaluated
16	2003	Not isolated	Nontypeable	MR	Unknown	Not evaluated
17	2003	Not isolated	Nontypeable	MR	Unknown	Not evaluated
18	2004	T	T	IR	Imipenem	Endogenous
19	2004	V	V	IR	Imipenem, levofloxacin	Endogenous
20	2004	Q	Q	IR	Imipenem	Endogenous
21	2004	R	R	IR	Imipenem	Endogenous
22	2004	A	A	IR	Imipenem	Endogenous
23	2004	U	J	IR	Imipenem	Exogenous
24	2004	S	B	MR	None	Exogenous
25	2004	Nontypeable	B	MR	None	Exogenous
26	2004	Not isolated	L	MR	None, ciprofloxacin	Exogenous
27	2004	Not isolated	B	MR	None	Exogenous
28	2004	Not isolated	Y	MR	None, ciprofloxacin	Indeterminate
29	2004	Not isolated	AA	MR	Imipenem, levofloxacin	Indeterminate
30	2004	Not isolated	Z	MR	Imipenem	Indeterminate

ICU admission, isolates were also typed by PFGE, which produced seven distinct genotypes. Six genotypes were isolated from individual patients only, and the remaining genotype (B) colonized six patients. Epidemiological analysis of these six patients with genetically related strains showed that none had previously been admitted to ICUs and only two patients presented some evidence of temporal clustering in the surgical ward.

DISCUSSION

This study explored the epidemiology of CR-PA in ICU patients and provided a detailed analysis of the main risk factors and presumed routes of digestive tract colonization in these patients. CR-PA was found in 16% of the ICU patients studied, 29% of whom presented colonization on ICU admission. These patients represented 31% of all those colonized with *P. aeruginosa*. Our incidence of CS-PA colonization was comparable to the rates of endemic digestive tract colonization found in other studies (16, 18).

In our study, acquisition of digestive tract CR-PA colonization was associated with similar risk factors already identified for carriage of other resistant bacteria in ICUs, but only severity of acute illness and, in particular, prior carbapenem and prior fluoroquinolone consumption presented independent associations.

Analysis of colonization routes has several potential limitations. First, patients may be colonized with multiple genotypes of *P. aeruginosa*; as only a single isolate was included in our genotyping analysis, it may not accurately represent the whole epidemiology. In addition, the stratification we used may have introduced a bias, and cultures of environmental sites and items from these ICU wards were not available. On balance, however, we think that it was a useful approach in this epidemiologic setting.

Colonization was endogenous in 33% of our patients, who showed pairs of clonally identical CS-PA and CR-PA isolates. All had been exposed to carbapenem therapy, and 80% showed an IR phenotype pattern. The risk of emergence of drug-resistant *P. aeruginosa* in the course of or after using antibiotics with high antipseudomonal activity, especially carbapenems, is well recognized (7, 12, 25).

On the other hand, 30% of our CR-PA carriers had an exogenous source and three clones were identified. Interestingly, we observed cross-transmission of clones J and L, whose index cases were among the endogenously acquired cases. Overall, the MR pattern prevailed in exogenously acquired colonizations; similar data were observed in another study (17), where 64% of the multidrug-resistant *P. aeruginosa* strains had been potentially transmitted via cross-colonization.

Although multiresistant *P. aeruginosa* strains have emerged worldwide in recent years, their prevalence and epidemiology have not yet been well established. An MR pattern was observed in 60% of our patients. Previous exposure to carbapenems was found in about 50% of these patients, and the influence of other antibiotics such as fluoroquinolones was also established. It has recently been postulated that fluoroquinolones may induce the expression of multidrug efflux pumps,

producing an MR *P. aeruginosa* phenotype (1, 2). The mechanisms of resistance in our patients were not studied, but five of the seven patients with CR-PA colonization who had been exposed to fluoroquinolones showed an MR pattern.

The evaluation of patients showing colonization at ICU admission deserves some comment. While more than half of the patients colonized on admission had a diversity of multiresistant clones which were not found during the study in patients with colonization acquired in ICUs, six patients showed the same multiresistant clone, B, which was responsible for five additional cases of colonization acquisition in the ICU. This alerted us to the fact that, concomitantly with our endemic CR-PA, our ICUs and other wards of the hospital also face a limited outbreak caused by this multiresistant clone.

Leaving aside the clone B outbreak, the CR-PA digestive tract colonization endemic in our ICUs was characterized by a polyclonal map of strains. The causes of the emergence of endogenous resistance in patients previously colonized by CS-PA may be considered the large-scale use of carbapenems and also the selection of intestinal flora, making the host more susceptible to colonization by resistant strains. In parallel, widespread use of fluoroquinolones may induce efflux systems and therefore render *P. aeruginosa* less susceptible to antibiotics (8, 13, 21). These conditions also increase the number of colonized patients and consequently the pressure of colonization, a circumstance that has been demonstrated to predispose to higher cross-transmission (5).

While maintaining vigilance and avoiding cross-transmission in our ICUs is mandatory, antibiotic control policies must be revised in the setting of endemic colonization or infection with CR-PA. Our study provides strong arguments for limiting carbapenem use and maybe fluoroquinolone use in the antibiotic policies of ICUs in order to prevent the worrying problem of emerging CR-PA. Because a program of extensive antimicrobial restriction can yield the potential development of new and possibly unexpected resistance patterns, efforts to encourage heterogeneity through individualization of drug use should be recommend.

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