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BRIEF REPORT

Risk factors for multidrug-resistant *Pseudomonas aeruginosa* acquisition. Impact of antibiotic use in a double case–control study

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Multidrug-resistant strains of *Pseudomonas aeruginosa* (MDRPA) have been increasing in some hospitals [1] and may become a public health problem [2].

The emergence of MDRPA has been related to exposure to antibiotics against *P. aeruginosa* [3, 4]. Most of these studies have focussed on particular environments such as the intensive care unit (ICU) [5] or particular antibiotic resistances, mainly quinolone-resistant *P. aeruginosa* and carbapenem-resistant *P. aeruginosa* or specific infection sites such ventilator-associated pneumonia or bacteraemia [6, 7]. Most studies have used case–control methodology or have investigated outbreaks, and the case–control studies have usually compared susceptibility to resistant micro-organisms. This methodology may overestimate the association between the resistance-defining antibiotic or may be falsely implicated as a potential risk factor for the acquisition of this pattern of susceptibility [8, 9].

The aim of this study was to assess the factors related to MDRPA acquisition, especially previous antibiotic exposure, using a double case–control methodology [10], analysing all types of infections and all hospital wards during a long period of follow-up.

We conducted a double case–control epidemiological study, exploring the risk factors (host characteristics, invasive procedures and, especially, previous antibiotic exposure) associated with the acquisition of MDRPA in hospitalised patients from 1 January 2001 to 31 December 2006 in a University Hospital with 450 beds. *P. aeruginosa* was isolated and identified by the microbiology laboratory by means of routine techniques. The susceptibility of

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isolates was determined by the MicroScan system (NC36 and NC38 panels) or the Kirby–Bauer method on Mueller–Hinton plates (bioMérieux, Marcy l'Etoile, France).

Case definition: patients with MDRPA, when the microorganism was resistant to all agents except colistin and/or amikacin. Control 1: patients with susceptible *P. aeruginosa* (SPA), when the microorganism was susceptible to all of the agents studied; and control 2: patients randomly selected among those admitted to the hospital during the same period with no positive cultures for *P. aeruginosa* and with a similar length of stay and severity index score to those with a positive culture for *P. aeruginosa*.

A bivariate analysis was performed to compare the characteristics of MDRPA patients with those of SPA and non-*P. aeruginosa* controls. *P*-values were calculated using the Chi-square test for categorical variables and the Mann–Whitney test for continuous variables. Predictors of nosocomial acquisition of MDRPA were assessed using logistic regression models. Two models were constructed, one using SPA as controls and another using patients without *P. aeruginosa* isolations as controls. Variables with a P-value<0.05 in the bivariate analysis were included in the logistic regression model. Statistical analyses were run using SPSS for Windows, rel. 12.0.0 (SPSS Inc., Chicago, Illinois).

During the study period, 1,403 incident *P. aeruginosa* isolates were identified in hospitalised patients; SPA: 532 (37.9%), MDRPA: 345 (24.6%) and *P. aeruginosa* with other susceptibility pattern 526 (37.5%). For study purposes, only the SPA and MDRPA patients and 690 patients without *P. aeruginosa* were included. The most common site of *P. aeruginosa* isolation was the respiratory tract (44.5%), followed by skin and soft tissue (21.6%), and the urinary tract (19.3%). The primary site of isolation was blood in only 3.9% of the study patients.

Table 1 shows the clinical and epidemiological characteristics of the cases and controls. In the univariate analysis, the most relevant data were that no statistically significant differences were found in the prevalence of most comorbidities, except for that of chronic obstructive pulmonary disease (COPD), which was highest in MDRPA. Mechanical ventilation, haemodialysis and bronchoscopy were more frequent in MDRPA than in SPA or control patients. Previous antibiotic therapy was significantly associated with a resistance pattern. The multivariate logistic regression analysis comparing controls without *P. aeruginosa* isolation with MDRPA showed that adjusted factors associated with an increased risk of MDRPA were male sex, more than three previous hospitalisations, simultaneous MDRPA isolates in the hospital, COPD, severity of illness, previous use of quinolones and carbapenems. When comparing MDRPA isolation versus SPA, the odds ratio (OR) for quinolones was much higher than that observed in the previous model, the OR for carbapenems was similar to that in the previous model and anti-*P. aeruginosa* penicillins were also a risk factor for MDRPA, while COPD disappeared (Table 2).

Several risk factors have been previously described to be associated to MDRPA acquisition, such as ICU stay, mechanical ventilation, higher severity index score, previous hospitalisations and co-morbidities (diabetes mellitus, renal failure, COPD and cystic fibrosis) [5, 6, 11]. In our study, only COPD, higher severity index score and previous hospitalisations were found to be a risk factor for MDRPA; these differences could be explained by the diverse settings in which the studies have been carried out.

The number of simultaneous detections of MDRPA, considered as a surrogate of colonisation pressure, emerged as a consistent independent factor associated with MDRPA; the role of colonisation pressure in the transmission of multidrug-resistant Gram-negative bacteria has not been well established [12]

One of the main concerns in the acquisition of resistance is previous antibiotic exposure. Our study shows that exposure to quinolones and carbapenems was associated with MDRPA acquisition in the adjusted analysis using the two control groups of patients. Several studies have reported that the emergence of MDRPA occurs after exposure to anti-pseudomonal antibiotics [5, 6]. Another study performed in critically ill patients with active surveillance to detect the colonisation of *P. aeruginosa* found that quinolones and anti-pseudomonal cephalosporins could prevent the acquisition of *P. aeruginosa* and that the use of these agents was not associated with the acquisition of resistance [13].

Most of the studies evaluating the risk of MDRPA acquisition have used the case–control methodology and have compared patients with resistant versus susceptible strains [14]. Selection of patients with susceptible organisms as controls overestimates the contribution of the resistance-defining antibiotic in the development of resistance [9, 10, 14]. This effect was also observed in the present study: when MDRPA were compared with susceptible *P. aeruginosa*, the adjusted ORs of MDRPA for exposure to quinolones and carbapenems were 15.3 and 3.5, respectively, and when MDRPA were compared with a control group without *P. aeruginosa* infection, the adjusted ORs were 1.8 and 2.3, respectively.

The present study has some limitations. First, the patient information was obtained retrospectively. The patients without *P. aeruginosa* were randomly selected from the same population as case patients and had similar length of stay and severity index score to those with MDRPA; as active surveillance was not carried out, we cannot ascertain that a proportion of control patients could be colonised by

Table 1 Clinical characteristics of the patients included in the study

	Non-PA, *n*=690 (44%)		SPA, *n*=532 (34%)		MDRPA, *n*=345 (22%)		*P*-value[1]	*P*-value[2]
Demographics								
Sex								
Male	375	(54.3)	316	(59.4)	250	(72.5)	<0.001	<0.001
Female	315	(45.7)	216	(40.6)	95	(27.5)		
Age (years)								
Mean (SD)	67.5	(16.8)	69.1	(16.6)	67.8	(13.5)	ns	0.011
Related to hospitalisation								
Previous hospitalisation								
None	358	(51.9)	283	(53.2)	119	(34.5)	<0.001	<0.001
1	151	(21.9)	112	(21.1)	59	(17.1)		
2	88	(12.8)	54	(10.2)	54	(15.7)		
≥3	93	(13.5)	83	(15.6)	113	(32.8)		
Previous ICU stay[3]	57	(8.4)	77	(14.8)	83	(24.3)	<0.001	<0.001
Length of hospital stay (days)	27.1	(15.4)	26.7	(20.9)	43.5	(31.7)	<0.001	<0.001
Days between admission and isolation	–	–	13.1	(11.0)	22.9	(18.9)	–	<0.001
Simultaneous MDRPA isolation	2.6	(2.2)	2.6	(2.3)	3.5	(2.1)	<0.001	<0.001
Comorbidities								
Diabetes	129	(18.7)	86	(16.2)	61	(17.7)	ns	ns
COPD	116	(16.8)	126	(23.7)	118	(34.2)	<0.001	0.001
Renal disease	69	(10.0)	54	(10.2)	40	(11.6)	ns	ns
Liver disease	92	(13.3)	36	(6.8)	37	(10.7)	ns	ns
HIV	33	(4.8)	16	(3.0)	12	(3.5)	ns	ns
Solid neoplasia	179	(25.9)	132	(24.8)	68	(19.7)	ns	ns
Haematologic neoplasia	23	(3.3)	18	(3.4)	11	(3.2)	ns	ns
Invasive procedures								
Mechanical ventilation	32	(4.6)	52	(9.8)	69	(20.0)	<0.001	<0.001
Haemodialysis	17	(2.5)	7	(1.3)	17	(4.9)	0.036	0.001
Bronchoscopy	44	(6.4)	46	(8.6)	44	(12.8)	0.001	0.050
Digestive endoscopy	80	(11.6)	37	(7.0)	27	(7.8)	ns	ns
Chemotherapy	19	(2.8)	8	(1.5)	7	(2.0)	ns	ns
Surgery	345	(50.0)	276	(51.9)	180	(52.2)	ns	ns
Severity								
Mean	2.6	(0.68)	2.8	(0.9)	3.1	(1.0)	<0.001	<0.001
1–3	612	(88.7)	393	(74.4)	193	(56.4)	<0.001	<0.001
4	78	(11.3)	135	(25.6)	149	(43.6)		
Previous antibiotic therapy								
No therapy	191	(27.7)	229	(43.0)	55	(15.9)		
Anti-*P. aeruginosa* drugs	240	(34.8)	60	(11.3)	209	(60.6)	<0.001	<0.001

Non-PA = non-*Pseudomonas aeruginosa*; SPA = susceptible *P. aeruginosa*; MDRPA = multidrug-resistant *P. aeruginosa*

Data are counts (%) or means (standard deviation, SD)

[1] Comparison between non-PA and MDRPA. The Chi-square test was used for categorical variables and the Mann–Whitney test for continuous variables

[2] Comparison between SPA and MDRPA. The Chi-square test was used for categorical variables and the Mann-Whitney test for continuous variables

[3] Twenty-six missing cases (12 control, 10 SPA, 4 MDRPA)

[4] 3M™ APR DRG (All-Patient Refined Diagnosis-Related Group)

Table 2 Multivariate analysis of risk factors for the isolation of multidrug-resistant *P. aeruginosa*

Factor			Non-PA vs. MDRPA[1]				SPA vs. MDRPA[2]			
			Crude OR	*P*-value	Adjusted OR (95% CI)	*P*-value	Crude OR	*P*-value	Adjusted OR (95% CI)	*P*-value
Sex										
	Female	Ref.	–	–	–	–	–	–	–	–
	Male		2.21	<0.001	1.67	0.004	1.80	<0.001	1.61	0.016
Previous Hospitalization				<0.001		<0.001		<0.001		<0.001
	None	Ref.	–	–	–	–	–	–	–	–
	1		1.18	0.386	1.30	0.226	1.25	0.246	1.57	0.065
	2		1.85	0.002	2.15	0.001	2.38	<0.001	2.86	<0.001
	≥3		3.66	<0.001	3.52	<0.001	3.24	<0.001	2.87	<0.001
Simultaneous MDRPA isolation										
	0	Ref.	–	–	–	–	–	–	–	–
	≥1		3.38	<0.001	3.47	<0.001	4.35	<0.001	3.91	<0.001
Severity Index										
	1–3	Ref.	–	–	–	–	–	–	–	–
	4		6.06	<0.001	4.29	<0.001	2.25	<0.001	1.63	0.020
Quinolones										
	No	Ref.	–	–	–	–	–	–	–	–
	Yes		2.21	<0.001	1.79	0.001	16.66	<0.001	15.25	<0.001
Carbapenems										
	No	Ref.	–	–	–	–	–	–	–	–
	Yes		4.14	<0.001	2.26	0.002	6.87	<0.001	3.53	<0.001
Anti-PA Penicillins										
	No	Ref.	–	–	–	–	–	–	–	–
	Yes		2.60	<0.001	1.09	0.816	5.52	<0.001	2.79	0.035
COPD										
	No	Ref.	–	–	–	–	–	–	–	–
	Yes		2.57	<0.001	2.02	<0.001	1.68	0.001	1.29	0.226

MDRPA = multidrug-resistant *P. aeruginosa*; non-PA = no positive culture for *P. aeruginosa*, control 1; SPA sensible *P. aeruginosa*, control 2

[1] Model adjusted by variables in the table, previous ICU stay, period, solid neoplasm, mechanical ventilation, haemodialysis, bronchoscopy and previous antibiotic therapy with cephalosporins and aminoglycosides. Monobactams and polymyxins, although significant at univariate analysis, were not included in the model because of sparse distribution data. Only significant factors at $P < 0.05$ are shown (except previous treatment with anti-PA penicillins)

[2] Model adjusted by variables in the table, previous ICU stay, period, liver disease, mechanical ventilation and previous antibiotic therapy with cephalosporins and aminoglycosides. Haemodialysis, monobactams and polymyxins, although significant at univariate analysis, were not included in the model because of sparse distribution data. Only significant factors at $P < 0.05$ are shown (except COPD)

MDRPA. The selection of the control groups is controversial; using patients without *P. aeruginosa* cannot differentiate what is a risk for MDRPA from what is a risk from *P. aeruginosa*, regardless of the susceptible profile. In the second model, we used patients with fully susceptible *P. aeruginosa*, because patients with many other non-MDRPA profiles is a very heterogeneous group. When a multivariate analysis including this group of patients was done, compared to the model with fully susceptible *P. aeruginosa*, the only risk factor that disappeared was the severity index score. The results may have been influenced by local epidemiological variables, such as possible environmental contamination with MDRPA, which is not applicable to other settings. In contrast, few studies have analysed the risk factors of resistance over such a long period, with a large number of patients with MDRPA and in a single hospital, including all hospital wards with a double case–control design. In conclusion, the present study suggests that, although many factors play a role in the acquisition of MDRPA, previous antibiotic exposure with quinolones and

carbapenems also play an important role in the acquisition of MDRPA. The results of our analysis suggest that greater efforts should be made to elucidate whether restricting the use of these antibiotics would help to control the acquisition of MDRPA.

References

1. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y (2006) Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. Antimicrob Agents Chemother 50:43–48
2. Falagas ME, Bliziotis IA (2007) Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? Int J Antimicrob Agents 29:630–636
3. Cao B, Wang H, Sun H, Zhu Y, Chen M (2004) Risk factors and clinical outcomes of nosocomial multi-drug resistant *Pseudomonas aeruginosa* infections. J Hosp Infect 57:112–118
4. Cipriano Souza R, Vicente AC, Vieira VV, Marques SG, Soares MG, Moura MC, Koifman RJ (2008) Clindamycin and metronidazole as independent risk factors for nosocomial acquisition of multidrug-resistant *Pseudomonas aeruginosa*. J Hosp Infect 69 (4):402–403
5. Paramythiotou E, Lucet JC, Timsit JF, Vanjak D, Paugam-Burtz C, Trouillet JL, Belloc S, Kassis N, Karabinis A, Andremont A (2004) Acquisition of multidrug-resistant *Pseudomonas aeruginosa* in patients in intensive care units: role of antibiotics with antipseudomonal activity. Clin Infect Dis 38:670–677
6. Harris AD, Smith D, Johnson JA, Bradham DD, Roghmann MC (2002) Risk factors for imipenem-resistant *Pseudomonas aeruginosa* among hospitalized patients. Clin Infect Dis 34(3):340–345
7. El Amari EB, Chamot E, Auckenthaler R, Pechère JC, Van Delden C (2001) Influence of previous exposure to antibiotic therapy on the susceptibility pattern of *Pseudomonas aeruginosa* bacteremic isolates. Clin Infect Dis 33:1859–1864
8. D'Agata EMC (2005) Methodologic issues of case–control studies: a review of established and newly recognized limitations. Infect Control Hosp Epidemiol 26:338–341
9. Harris AD, Samore MH, Lipsitch M, Kaye KS, Perencevich E, Carmeli Y (2002) Control-group selection importance in studies of antimicrobial resistance: examples applied to *Pseudomonas aeruginosa*, *Enterococci*, and *Escherichia coli*. Clin Infect Dis 34 (12):1558–1563
10. Zavascki AP, Cruz RP, Goldani LZ (2005) Risk factors for imipenem-resistant *Pseudomonas aeruginosa*: a comparative analysis of two case–control studies in hospitalized patients. J Hosp Infect 59(2):96–101
11. Lieberman D, Lieberman D (2003) Pseudomonal infections in patients with COPD: epidemiology and management. Am J Respir Med 2:459–468
12. Harris AD, McGregor JC, Furuno JP (2006) What infection control interventions should be undertaken to control multidrug-resistant gram-negative bacteria? Clin Infect Dis 43(Suppl 2): S57–S61
13. Martínez JA, Delgado E, Martí S, Marco F, Vila J, Mensa J, Torres A, Codina C, Trilla A, Soriano A, Alquezar A, Castro P, Nicolás JM (2009) Influence of antipseudomonal agents on *Pseudomonas aeruginosa* colonization and acquisition of resistance in critically ill medical patients. Intensive Care Med 35:439–447
14. Kaye KS, Harris AD, Samore M, Carmeli Y (2005) The case–case–control study design: addressing the limitations of risk factor studies for antimicrobial resistance. Infect Control Hosp Epidemiol 26:346–351