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Emergence of Antibiotic-Resistant *Pseudomonas aeruginosa*: Comparison of Risks Associated with Different Antipseudomonal Agents

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***Pseudomonas aeruginosa* is a leading cause of nosocomial infections. The risk of emergence of antibiotic resistance may vary with different antibiotic treatments. To compare the risks of emergence of resistance associated with four antipseudomonal agents, ciprofloxacin, ceftazidime, imipenem, and piperacillin, we conducted a cohort study, assessing relative risks for emergence of resistant *P. aeruginosa* in patients treated with any of these drugs. A total of 271 patients (followed for 3,810 days) with infections due to *P. aeruginosa* were treated with the study agents. Resistance emerged in 28 patients (10.2%). Adjusted hazard ratios for the emergence of resistance were as follows: ceftazidime, 0.7 ($P = 0.4$); ciprofloxacin, 0.8 ($P = 0.6$); imipenem, 2.8 ($P = 0.02$); and piperacillin, 1.7 ($P = 0.3$). Hazard ratios for emergence of resistance to each individual agent associated with treatment with the same agent were as follows: ceftazidime, 0.8 ($P = 0.7$); ciprofloxacin, 9.2 ($P = 0.04$); imipenem, 44 ($P = 0.001$); and piperacillin, 5.2 ($P = 0.01$). We concluded that there were evident differences among antibiotics in the likelihood that their use would allow emergence of resistance in *P. aeruginosa*. Ceftazidime was associated with the lowest risk, and imipenem had the highest risk.**

Pseudomonas aeruginosa is a leading cause of nosocomial infections, ranking second among the gram-negative pathogens reported to the National Nosocomial Infection Surveillance System. There are a limited number of antimicrobial agents with reliable activity against *P. aeruginosa*, including antipseudomonal penicillins and cephalosporins, carbapenems, and fluoroquinolones, particularly ciprofloxacin. Aminoglycosides are frequently used as part of combination regimens for treatment of serious pseudomonal infections but are generally not recommended as single drugs. For each of these agents, emergence of resistance during therapy has been described and has been recognized as a cause of treatment failure (4, 6, 8, 10, 12, 13). Yet comparative analyses of the frequency of emergence of resistance associated with different classes of antipseudomonal drugs are lacking, even though knowledge about the relative risks of emergence of resistance with different antibiotics could be useful in helping to guide therapeutic choices. Ideally, the information regarding risks of emergence of resistance associated with individual regimens should come from a large-scale prospective randomized study with multiple treatment groups. Unfortunately, the costs associated with such a study would be prohibitive. Therefore, we performed an observational study to compare the relative risks for emergence of resistant *P. aeruginosa* associated with four individual antipseudomonal agents.

In order to directly compare the overall effect of each antibiotic, we examined emergence of resistance to any antipseudomonal antibiotic. In a secondary analysis, individual antibiotic resistances were studied as separate and distinct endpoints, focusing on the association between emergence of resistance to a specific agent and exposure to that agent.

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MATERIALS AND METHODS

Hospital setting, data collection, and microbiology. The Deaconess Hospital is a 320-bed urban tertiary-care teaching hospital in Boston, Mass. It utilizes 24 intensive-care unit (ICU) beds and has approximately 11,000 patients admitted per year.

The study was designed as a historical cohort study of data prospectively collected in the hospital repository. Data were collected from administrative, pharmacy, and laboratory computerized databases by using a relational database management system (Access; Microsoft Corp., Redmond, Wash.). The presence of infections was confirmed by reviewing the medical records and laboratory, pathology, and radiology results.

P. aeruginosa had been identified from clinical specimens submitted to the microbiology laboratory by using Gram-Negative Identification Panel Type II (Dade International Inc., West Sacramento, Calif.). Susceptibility had been determined by microdilution broth testing (MicroScan; Dade International Inc.).

Definitions and study design. Four antipseudomonal drugs used frequently in our hospital were studied: ceftazidime, ciprofloxacin, imipenem, and piperacillin. Piperacillin-tazobactam was used infrequently for treating pseudomonal infections and was grouped with piperacillin.

Criteria for entry into the study population were as follows: (i) admission between 1 August 1994 and 31 July 1996 with a hospital stay of at least 2 days; (ii) recovery of *P. aeruginosa* from clinical culture; (iii) susceptibility of the first pseudomonal isolate to at least one of the four antibiotics listed above; (iv) subsequent treatment with at least one of these drugs; and (v) confirmation of clinical infection on the basis of Centers for Disease Control and Prevention definitions for infection (modified to include community infections and with exclusion of asymptomatic bacteriuria) (7). The patients were followed from the date of detection of their baseline isolate until discharge or until the detection of the emergence of resistance in a subsequent clinical isolate of *P. aeruginosa*.

The emergence of resistance to individual antibiotics was also studied. Patients were included in each of these analyses according to the susceptibility of their baseline isolate to the study drug. The follow-up periods were defined as described above, except that the endpoint was considered to be the emergence of resistance to the specific antibiotic that defined the cohort; e.g., for the cohort defined by baseline susceptibility to ceftazidime, the outcome was the emergence of resistance to ceftazidime.

The MICs determining susceptibility thresholds for the different drugs were as follows: $\leq$64 μg/ml for piperacillin, $<$16 μg/ml for ceftazidime, $<$ 8 μg/ml for imipenem, and $<$ 2 μg/ml for ciprofloxacin. Isolates with intermediate susceptibility were considered resistant in order to match better treatment decisions in clinical settings. The emergence of resistance was defined as the clinical detection of resistant *P. aeruginosa* with a minimum fourfold increase (two dilutions) in MIC compared to that of the baseline isolate (the first isolate for each admission), which resulted in a change in the interpretive criteria.

To explore confounding factors, the following variables were analyzed in

addition to the study drugs: age, gender, underlying diseases and weighted comorbidities (Charlson comorbidity score) (3), culture site, surgical procedures, ICU stays, time interval between hospital admission and the detection of the baseline isolate, number of initial antibiotic resistances in the baseline isolate, average number of nursing hours per day (calculated from nursing administrative records), administration of more than one study drug, and concomitant administration of aminoglycosides. A score was constructed in order to adjust for the intensity of clinical culturing by calculating the average number of cultures obtained per day during the follow-up period.

All available pairs of isolates from patients in whom resistance emerged were studied by pulsed-field gel electrophoresis (PFGE) as previously described (5). Isolates from before and after the emergence of resistance were compared.

Statistical analysis. Statistical analyses were performed with SAS (SAS Institute Inc., Cary, N.C.) and Stata (Stata Corp., College Station, Tex.) software. Survival analysis was performed in order to allow for different follow-up periods.

Crude and multivariable Cox proportional-hazard models were used to address the emergence of resistance. Thus, for each day of follow-up, comparisons were made only among individuals who were still in the hospital on that day. Treatment courses with the study antibiotics were analyzed as time-dependent variables. The measure of relative risk in Cox proportional-hazard regression is the hazard ratio (HR), which in this study represents the risk ratio per unit of time for emergence of resistance, comparing "exposed" and "unexposed" patients (e.g., patients who received ceftazidime versus patients who did not receive ceftazidime).

Variables with a P value of <0.2 in the crude analysis were considered candidates for multivariable analysis and added to a model that included the study drugs. In addition, variables were tested for confounding by adding them one at a time to the model and examining their effects on the beta coefficients of the study drugs. Variables which caused substantial confounding (a change in the beta coefficient of greater than 10%) were included in the final model. Effect modification between antibiotics was examined by using interaction terms. The proportional-hazard assumption was tested for every analyzed variable.

All statistical tests were two tailed. A P value of <0.05 was considered significant.

RESULTS

Emergence of resistance (to any of four antipseudomonal drugs). Two hundred and seventy-one patients satisfied the criteria for entry in the study cohort; 162 were males. The single most common site of infection was a wound. The average age of the patients was 62 (range, 24 to 94). The susceptibility pattern of the baseline isolates was as follows: 15 (5%) were resistant to piperacillin, 19 (7%) were resistant to ceftazidime, 36 (13%) were resistant to imipenem, and 58 (21%) were resistant to ciprofloxacin. One hundred and eighty-five (68%) of the baseline isolates were susceptible to all four study drugs, 56 (20%) were resistant to one agent, 27 (10%) were resistant to two agents, and 6 (2%) were resistant to three agents. The patients were followed for a total of 3,810 days. The median follow-up period was 11 days (range, 2 to 72 days). The number of patients receiving each of the study antibiotics was as follows: imipenem, $n = 37$ (14%); ciprofloxacin, $n = 98$ (36%); piperacillin, $n = 91$ (33%); ceftazidime, $n = 125$ (46%). Sixty-six patients received more than one study agent. Seventy-seven patients received an aminoglycoside in addition to a study agent. The median duration of aminoglycoside therapy was 6 days.

P. aeruginosa resistant to at least one of the study agents emerged in 28 patients (10.2%), an incidence of 7.4 per 1,000 patient-days. Pairs of isolates (before and after resistance emerged) from nine of these patients were available and were typed by PFGE. Typing patterns before and after the emergence of resistance were identical in each of the patients, confirming that resistance emerged in a susceptible isolate. The median time to emergence of resistance was 14 days (range, 2 to 65 days). Characteristics and exposures in these patients are summarized in Table 1 along with HRs. Imipenem treatment was associated with a 2.9-fold-higher hazard of emergence of resistance. In the crude analysis, other factors significantly associated with emergence of resistance were the frequency of microbiological culturing and the length of the hospital stay before entry into the cohort.

The multivariable analysis included the four study antibiotics, other variables that were independently significant (e.g., frequency of microbiological culturing), and factors which were considered clinically significant and which were putative confounders (e.g., aminoglycoside use and ICU exposure) (Table 2). Treatment with imipenem was still significantly associated with the emergence of resistance (adjusted HR = 2.8; $P = 0.02$).

Combination therapy with an aminoglycoside did not appear to prevent the emergence of resistance ($P = 0.8$ in the multivariable model). The relatively infrequent use of aminoglycosides in this study reflected a reluctance by clinicians to prescribe aminoglycosides for patients whose risk for aminoglycoside-induced nephrotoxicity was high because of age and underlying comorbidities, such as preexisting renal disease. The analysis showed that there was confounding of the effect of aminoglycosides because of preferential use of aminoglycosides in patients who were at higher risk for emergence of resistance. This confounding was manifested by the decrease in relative risk for aminoglycoside exposure from 1.3 in the crude analysis to 0.8 in the adjusted model.

We also examined the emergence of resistance to each individual drug, first by examining crude relative risks and then by adjusting for aminoglycoside use and culturing score. The crude and adjusted relative risks were very similar. Resistance to ceftazidime was detected in 14 patients, 6 of whom had been treated with ceftazidime and 8 of whom had been treated with other antipseudomonal agents (association between ceftazidime treatment and ceftazidime resistance, adjusted HR = 0.8 [$P = 0.7$]). Resistance to ciprofloxacin developed in 12 patients, 11 of whom had been treated with ciprofloxacin and 1 had been treated with other antipseudomonal agents (association between ciprofloxacin treatment and ciprofloxacin resistance, adjusted HR = 9.2 [$P = 0.04$]). Emergence of resistance to imipenem occurred in eight patients, seven of whom had been treated with imipenem (association between imipenem treatment and imipenem resistance, adjusted HR = 44 [$P = 0.001$]). Emergence of resistance to piperacillin occurred in 11 patients, 7 of whom had been treated with piperacillin (association between piperacillin treatment and piperacillin resistance, adjusted HR = 5.2 [$P = 0.01$]).

DISCUSSION

Resistance to antimicrobial agents is an increasing clinical problem and is a recognized public health threat. *P. aeruginosa* shows a particular propensity for the development of resistance. The emergence of resistance in *P. aeruginosa* also limits future therapeutic choices and is associated with increased rates of mortality and morbidity and higher costs (2, 8). Therefore, we conducted this study to assess resistance arising during treatment with different antibiotics, first by examining the overall effect of each antibiotic on emergence of resistance and second by examining the emergence of resistance to individual agents.

We found that emergence of resistance to at least one antibiotic occurred in 10.2% of the patients (7.4 cases per 1,000 patient-days). This proportion should be considered a minimum estimate of the risk of emergence of resistance during antipseudomonal therapy, since it is based solely on clinical cultures and includes follow-up only during the index hospitalization. Resistance emerged during treatment with each class of antibiotic and did not appear to be significantly prevented by the use of combination therapy with aminoglycosides. However, the latter issue needs to be further examined

TABLE 1. Clinical and microbiological characteristics, exposures, and Cox proportional HRs for the emergence of resistance to any of the four study drugs

Characteristic (n = 271)	Value	HR (95% CI)[a]	P value
Demographics			
Age (yr); mean (SD)	62 (15.6)	1.0 (0.98–1.03)	0.7
Male gender; no. (%)	162 (59)	1.1 (0.5–2.5)	0.7
Underlying conditions			
AIDS; no. (%)	16 (6)	0.9 (0.1–6.7)	0.9
Cancer; no. (%)	42 (15)	1.2 (0.4–3.0)	0.8
Diabetes; no. (%)	145 (53)	0.8 (0.4–1.8)	0.7
Solid-organ transplant; no. (%)	29 (11)	1.2 (0.4–3.1)	0.7
Chronic lung disease; no. (%)	38 (14)	0.7 (0.2–2.3)	0.6
Charlson score; mean (SD)	3.4 (1.8)	1.0 (0.8–1.3)	1.0
Exposures during admission			
Transfer from institution; no. (%)	52 (19)	1.5 (0.7–3.4)	0.3
ICU stay; no. (%)	76 (28)	0.6 (0.3–1.4)	0.22
Surgical procedure; no. (%)	127 (46)	0.8 (0.4–1.8)	0.6
Nursing index[b]; mean (SD)	58.4 (25.7)	1.0 (0.99–1.01)	0.9
Culturing score; no. (%)	97 (35)	2.6 (1.1–5.7)	0.027
Rx with study agent[d]; no. (%)			
Ceftazidime	125 (46)	0.6 (0.3–1.4)	0.25
Ciprofloxacin	98 (36)	0.9 (0.4–2.1)	0.9
Imipenem	37 (14)	2.9 (1.3–6.4)	0.008
Piperacillin	91 (33)	1.4 (0.6–3.1)	0.4
Rx with >1 study agent[d]; no. (%)	66 (24)	1.4 (0.6–3.1)	0.4
Rx with aminoglycosides; no. (%)	77 (28)	1.3 (0.6–2.7)	0.6
Baseline isolate			
Hospital days before culture; mean (SD)	4.3 (8.0)	1.02 (0.99–1.05)	0.07
Nosocomial isolate; no. (%)	90 (33)	1.2 (0.6–2.7)	0.7
Number of resistances[e]; mean (SD)	0.47 (0.76)	1.2 (0.8–2.0)	0.4
Culture site; no. (%)			
Blood	14 (5.1)	0.5 (0.1–4.0)	0.5
Effusion fluid	13 (4.7)	2.4 (0.7–8.2)	0.15
Respiratory tract	61 (22)	0.8 (0.3–1.9)	0.6
Urine	40 (15)	1.1 (0.3–3.7)	0.9
Wound	129 (47)	1.1 (0.5–2.4)	0.8
Other	16 (5.8)	0.8 (0.2–3.7)	0.8

[a] CI, confidence interval.
[b] Total nursing hours/number of days inside the hospital.
[c] Number of cultures from the baseline isolate/days of follow-up (dichotomized at mean).
[d] Time-dependent variable (see text); Rx, treatment.
[e] The baseline isolate could be already resistant to zero, one, two, or three of the four study drugs.

in studies that include larger number of patients on aminoglycosides.

Important differences between antibiotics were evident. First, imipenem was associated with a significantly higher overall risk of emergence of resistance (HR, 2.8; $P = 0.02$) and had the strongest association with emergence of resistance to itself in the analysis of individual antibiotics (HR, 44; $P = 0.001$). In contrast, ceftazidime had the lowest risk for emergence of resistance in the combined analysis (HR, 0.7), and showed no association with emergence of resistance to itself (i.e., to ceftazidime) (HR, 0.8; $P = 0.7$). The relative risk for ciprofloxacin in the combined analysis was also low, but in contrast to ceftazidime, ciprofloxacin was distinctly associated with emergence of resistance to itself (HR, 9.2; $P = 0.04$).

The finding that imipenem carries a higher risk of emergence of pseudomonal resistance is consistent with the results of other studies. The clinical emergence of resistant *P. aeruginosa* has been described during imipenem therapy, ranging from 14 to 53% and occasionally leading to treatment failures (4, 6, 9, 13, 14). In two randomized clinical trials, these rates were significantly higher for imipenem than for ciprofloxacin or piperacillin-tazobactam (6, 9). Moreover, imipenem resistance in *P. aeruginosa* became widespread in some hospitals soon after the introduction of this agent (1). In this study more patients with *P. aeruginosa* infections were included than in the randomized trials, and adjustment for possible confounding was performed. Moreover, this study allowed direct compari-

TABLE 2. Multivariable Cox hazard models for the emergence of resistance to any of the four study drugs[a]

Antibiotic	Events (no./total Rx)	Multivariable model	
		HR (95% CI)	P value
Culturing score	NI	2.5 (1.1–6.0)	0.04
Aminoglycosides	13/77	0.8 (0.4–2.0)	0.8
Ceftazidime	10/125	0.7 (0.3–1.7)	0.4
Ciprofloxacin	12/98	0.8 (0.3–2.0)	0.6
Imipenem	11/37	2.8 (1.2–6.6)	0.02
Piperacillin	9/91	1.7 (0.7–4.1)	0.3

[a] Rx, treatment; CI, confidence interval; NI, not included.

son of four different antipseudomonal agents while accounting for differences in follow-up periods by using survival analysis. Although treatment with imipenem could result more often in the emergence of resistant *P. aeruginosa* than treatments with other antipseudomonal agents, this tendency may not translate into a higher prevalence of imipenem resistance among hospital isolates. Ciprofloxacin resistance, for instance, is more common than imipenem resistance in *P. aeruginosa* isolated from inpatients at our institution (15 versus 9%) (14). This apparent discrepancy might be related to differences in the frequency of use of various agents and to the different likelihoods of persistence of resistant strains.

An observational study such as this has a number of limitations, including the potential for confounding due to the lack of randomization and the use of more than one study antibiotic in some patients. However, multivariable analysis allowed us to adjust for confounding variables and to assess the independent effects of each antibiotic.

Follow-up was continued only during the hospital stay, and not until the resolution of infection. We used survival analysis to avoid the potential bias relating to differences in follow-up. Using these methods, the risks for patients with the same follow-up were compared to each other. All of the patients had follow-up cultures, but the frequency of culturing differed among patients. Although we adjusted for the differences in culturing density by adjusting for the culturing score, the confounding related to it may not have been fully controlled for. Our study also did not specifically address the mechanisms by which resistance occurred (11). We typed only a limited number of organisms by PFGE to show that resistance emerged in a susceptible strain. Knowledge of the specific means by which resistant microorganisms emerge is likely to be useful for designing effective prevention measures.

One should always remember that the spread of resistant organisms from patient to patient can be reduced by appropriate infection control measures. The results of this study are not generalizable to organisms other than *P. aeruginosa*. In other gram-negative pathogens, such as *Enterobacter* spp., emergence of resistance to broad-spectrum cephalosporins, including ceftazidime, may occur frequently, while resistance to imipenem is extremely rare.

In conclusion, the present study is important from a practical point of view. We believe that the use of imipenem for treatment of *P. aeruginosa* should be reserved for situations where the infection is polymicrobial, particularly when anaerobic bacteria are present, or for pseudomonal isolates resistant to other antibiotics. In cases where imipenem is selected as the antipseudomonal antibiotic, the potential for emergence of resistance should be anticipated, and in appropriate circumstances, routine culturing and susceptibility testing should be performed to detect the emergence of resistance *P. aeruginosa* as soon as possible.

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