Influence of Previous Exposure to Antibiotic Therapy on the Susceptibility Pattern of *Pseudomonas aeruginosa* Bacteremic Isolates

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Many patients who present with *Pseudomonas aeruginosa* bacteremia have been previously exposed to antibiotics. To assess whether resistance of bacteremic strains to antipseudomonal antibiotics (piperacillin, ceftazidime, imipenem, ciprofloxacin, or aminoglycosides) is associated with previous exposure to these drugs, a case-control study including 267 cases of *P. aeruginosa* bacteremia was conducted. Twenty-five percent of the episodes had been preceded by the exposure to an antipseudomonal antibiotic. Eighty-one strains were resistant to at least 1 antibiotic; 186 were susceptible to all drugs. Via univariate analysis, the risks of resistance to ceftazidime and imipenem were found to be significantly associated with previous receipt of these agents. Using multivariate analysis, exposure to any antipseudomonal antibiotic as a monotherapy was found to be associated with an increased risk of subsequent resistance to itself (odds ratio, 2.5; $P = .006$). Therefore, clinicians should avoid readministering previously prescribed antibiotics when initiating empiric therapies for possible *P. aeruginosa* bacteremia, especially when they have been given as monotherapies.

Pseudomonas aeruginosa is a leading cause of nosocomial bloodstream infections, ranking third among gram-negative bacteria, after *Escherichia coli* and *Klebsiella* species [1]. Despite improvement in recent years, the prognosis of *P. aeruginosa* bacteremia remains poor, with case-fatality rates of ⩾20% [2–5]. Factors that delay therapeutic improvements are the rapid course of the disease, the scarcity of antibiotics with antipseudomonal activity, and the ease with which the bacte-

rium acquires new resistances during antibiotic therapy [6]. As a result of this capacity to rapidly acquire resistance mechanisms, *P. aeruginosa* bacteremia frequently follows other infections treatment with antibiotic regimen that include antipseudomonal drugs.

Because the symptoms of *P. aeruginosa* bacteremia are nonspecific, the initial antibiotic therapy for possible *P. aeruginosa* bacteremia is almost always empirical, with a pending identification of the responsible pathogen and an unknown antibiotic resistance profile. Inappropriate antibiotic treatment of bacteremia is associated with a significantly poorer outcome [7]. It is therefore important to determine whether recent exposure to antibiotics with antipseudomonal activity increases the risk of resistance of bacteremic strains to these agents [3]. Answering this question would help clinicians choose the most adequate empirical treatment in clinical situations that include *P. aeruginosa* bacteremia as a possible cause.

We identified 267 *P. aeruginosa* bacteremic events that occurred at a tertiary-care hospital and conducted

a case-control study to determine whether recent exposure to antibiotics with antipseudomonal activity was associated with an increased risk of resistance toward these drugs.

PATIENTS AND METHODS

This study took place at the Geneva University Hospital, Switzerland, a 1000-bed tertiary-care teaching center that serves as a first-line medical center for an urban population of ∼400,000 inhabitants and as a referral center for a larger population coming from both Switzerland and nearby France.

Study design. We used the microbiology laboratory database of the hospital to identify all inpatients that had had 1 or several blood cultures positive for *P. aeruginosa* from 1 January 1989 through 31 December 1998. Medical records were reviewed for information on demographic characteristics, clinical presentation of bacteremia, primary site of *P. aeruginosa* infection, underlying medical conditions, immunosuppression at the time of bacteremia, invasive procedures, laboratory results of antibiotic susceptibility, and recent antibiotic treatment including an agent with antipseudomonal activity. An "episode" of *P. aeruginosa* bacteremia was defined as a positive blood culture with this pathogen. "Previous monotherapy" was defined as a therapy that started within 30 days before the positive blood culture, included only 1 of the antipseudomonal antibiotics used in our institution (piperacillin, ceftazidime, imipenem, ciprofloxacin, gentamicin, and amikacin), was administered for >48 h, and was stopped <15 days before the bacteremia. "Previous combination therapy" was defined as follows: treatment was initiated within 30 days and stopped <15 days before the positive blood culture; it included the concomitant use for ≥48 h of a β-lactam antibiotic, and either an aminoglycoside or ciprofloxacin, or the combination ciprofloxacin plus aminoglycoside, with an exposure to a single drug for no more than 48 h. All other patterns of antibiotic exposure were classed in the "no previous treatment" group. In a first analysis, "cases" were defined as episodes of bacteremia caused by a strain of *P. aeruginosa* resistant to at least 1 antipseudomonal antibiotic and "controls" as episodes involving strains susceptible to all 6 antibiotics. Three categories of exposure were examined: any previous combination therapy, any previous monotherapy, and no previous therapy. In subsequent analyses, cases were episodes caused by a strain resistant to 1 specified antibiotic, and controls were episodes caused by a strain susceptible to that same antibiotic; hence, bacteremic strains resistant to ≥2 antibiotics contributed more than once to these analyses. Patients were considered exposed if they had received the specified antibiotic either as a monotherapy or as an agent included in a combination therapy.

Microbiology. A minimum of 2 pairs of blood cultures was performed at the time of presumed bacteremia. *P. aeru-*

ginosa was identified at the laboratory of clinical microbiology via standard clinical microbiology methods [8]. Antimicrobial susceptibility was determined by disk diffusion methods according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [9]. An isolate was considered susceptible, intermediate, or resistant according to the criteria of the NCCLS. The isolates with intermediate susceptibility were classified as resistant for analysis. No molecular typing was performed.

Statistical analysis. We calculated crude ORs and exact 95% CI to evaluate the potential relation between previous antipseudomonal therapy and resistance of the bacteremic strain. Two-tailed Fisher's exact tests were used for the comparison of proportions.

Multiple logistic regression was used to assess whether having received a previous monotherapy or combination therapy including a specific antipseudomonal agent were independent risk factors for resistance of the bacteremic isolate to that same agent. Because data were scarce, observations corresponding to all 6 antibiotics were pooled in a stratified model. Therefore, each episode of bacteremia contributed 5 times to the model (1 stratum per antibiotic); variance estimates were adjusted to reflect the resulting dependence among observations. Previous monotherapy and combination therapy were forced in the model; we further considered independent variables with $P <$ .2 in univariate logistic regression (data not shown). To limit the risk of overfitting, no interaction terms were tested. Regression analyses were performed by STATA version 6.0 (STATA Corporation).

RESULTS

Characteristics of bacteremic episodes. During the study period, 273 *P. aeruginosa* bacteremic episodes occurred in 267 patients. Computerized microbiological data and medical records were available for all patients. Four patients had 2 independent episodes of bloodstream infections separated by 14–69 days, and 1 person had 3 episodes over a period of 50 days. We report here on the 267 initial episodes of *P. aeruginosa* bacteremia.

The overall incidence of *P. aeruginosa* bacteremia at the Geneva University Hospital was 1 per 1000 admissions (range per year, 0.63‰–1.45‰). *P. aeruginosa* bacteremia accounted for 5.4% (range, 3.7%–7.6%) of all bloodstream infections. The mean age of the patients was 59 years (range, 1 day to 93 years; SD, 22 years), and approximately two-thirds were men (table 1). At the time of bacteremia, 32% of patients were hospitalized in acute-care and 14% in chronic-care medical services, 17% were in surgical wards, and 37% were in intensive care units (medical and surgical). The most common sites of primary infection were the respiratory and the urinary tracts; no source

Table 1. Univariate associations of clinical characteristics with resistance of *Pseudomonas aeruginosa* in 267 patients.

| Characteristic | Total no. (%) | Resistance to antipseudomonal agents | | OR (95% CI) | *P* |
		≥1 resistance (cases; *n* = 81)	No resistance (controls; *n* = 186)		
Previous antipseudomonal therapy[a]					
No	201 (75.3)	55	146	—	—
Yes	64 (24.0)	26	38	1.8 (0.96–3.4)	.09
Monotherapy	54 (20.2)	22	32	1.8 (0.92–3.6)	.07
Combination therapy[b]	10 (3.7)	4	6	1.8 (0.35–7.8)	.47
Age, years					
<65	132 (49.4)	44	88	—	—
≥65	135 (50.6)	37	98	0.76 (0.43–1.3)	.35
Sex					
Male	195 (73.0)	59	136	—	—
Female	72 (27.0)	22	50	1.0 (0.53–1.9)	1.0
Calendar time					
1988–1992	126 (47.2)	27	99	—	—
1993–1998	141 (52.8)	54	87	2.3 (1.3–4.1)	.003
Ward					
Medical acute care	84 (31.5)	18	66	—	—
Other	183 (68.5)	63	120	1.9 (1.0–3.7)	.03
Primary site(s) of infection					
Unknown/other	142 (53.2)	38	104	—	—
Respiratory	47 (17.6)	16	31	1.4 (0.64–3.0)	.36
Urinary	46 (17.2)	20	26	2.1 (1.0–4.4)	.04
Digestive	24 (9.0)	9	15	1.6 (0.58–4.4)	.33
Vascular	17 (6.4)	3	14	0.59 (0.10–2.3)	.56
Cutaneous	13 (4.9)	2	11	0.50 (0.05–2.4)	.52
Clinical presentation					
Fever/simple sepsis	183 (68.5)	48	135	—	—
Severe sepsis	29 (10.9)	11	18	1.7 (0.68–4.2)	.26
Shock	55 (20.6)	22	33	1.9 (0.94–3.7)	.06
Underlying medical condition[c]					
No	34 (12.7)	11	23	—	—
Yes	233 (87.3)	70	163	0.9 (0.39–2.2)	.84
Immunological risk factor for infection					
None of the following	203 (76.0)	64	139	—	—
Neutropenia	48 (18.0)	10	38	0.57 (0.24–1.3)	.16
Steroid treatment	18 (6.7)	7	11	1.4 (0.43–4.1)	.60
Invasive procedures					
None of the following	48 (18.0)	7	41	—	—
Vascular catheter	205 (76.8)	68	137	2.9 (1.2–8.1)	.01
Urinary catheter	150 (56.2)	50	100	2.9 (1.2–8.3)	.02
Intubation	101 (37.8)	32	69	2.7 (1.0–7.5)	.03
Drainage tube	57 (21.3)	17	40	2.5 (0.86–7.8)	.10
Parenteral nutrition	38 (14.2)	14	24	3.4 (1.1–11.3)	.02
Other	95 (35.6)	31	64	2.8 (1.1–7.8)	.02

[a] Includes ceftazidime, piperacillin, imipenem, ciprofloxacin, an aminoglycoside, or some combination of these; 2 patients had received a combination therapy with 2 antipseudomonal agents preceded or followed by a monotherapy with a third agent.
[b] Two patients who had received monotherapy followed by combination therapy including other drugs were classified as having received combination therapy.
[c] Malignancy, AIDS, diabetes, pancreatitis, respiratory dysfunction, heart disease, renal failure, severe nonpseudomonal infection, or severe trauma.

of bacteremia could be identified in about half the patients. Severe sepsis was the initial manifestation of bloodstream infection in 11% of episodes and shock in 22%. Approximately 233 (90%) of 267 patients had severe underlying medical conditions: 91, malignancy (34%); 69, heart disease (26%); 58, respiratory dysfunction (22%); 51, nonpseudomonal severe infection (19%); 48, renal failure (18%); 29, diabetes (11%); 16, AIDS (6%); 14, severe trauma (5%); and 12, pancreatitis (4%). Immunosuppression was documented in 64 patients (24%) and invasive procedure that increased the risk of bacteremia in 219 (82%).

Sixty-six patients (24.7%) had been exposed to an antibiotic therapy active against *P. aeruginosa* (monotherapy, $n = 54$; combination therapy, $n = 8$; monotherapy followed by a combination therapy with 2 other agents, $n = 2$; table 1) before the bacteremic event. Of these regimens, 36 included imipenem; 9, piperacillin; 9, ciprofloxacin; 8, ceftazidime; and 32, an aminoglycoside (table 2). Of the 267 *P. aeruginosa* blood isolates, 15 were not tested for susceptibility to imipenem and 1 isolate was not tested for susceptibility to piperacillin. A total of 186 blood isolates (70%) were susceptible to all tested antibiotics, 35 (13%) were resistant to 1 antibiotic, 27 (10%) to 2 antibiotics, and 19 (7%) to $\geqslant 3$ antibiotics. Forty-three isolates (16%) were resistant to an aminoglycoside (gentamicin or amikacin), 41 (15%) to imipenem, 29 (11%) to piperacillin, 16 (6%) to ceftazidime, and 15 (6%) to ciprofloxacin.

Univariate risk factors for resistance to $\geqslant 1$ antipseudomonal agent. Patients who had been exposed to previous therapy including an antipseudomonal agent were marginally more likely to have experienced a *P. aeruginosa* bloodstream infection with a strain resistant to $\geqslant 1$ of the study antibiotics than patient who had not been previously exposed (OR, 1.8; 95% CI, 1.0–3.4; $P = .06$; table 1). Previous exposure to a monotherapy was marginally associated with an increased risk of resistance (OR, 1.8; 95% CI, 0.92–3.6; $P = .07$). No statistically significant association was found between a previous combination therapy and risk of resistance (OR, 1.8; 95% CI, 0.35–7.8; $P = .47$). Bacteremia experienced between 1993 and 1998, hospitalization on other units than the acute-care medical services, urinary source of infection, septic shock as clinical mode of presentation ($P = .06$), and having experienced an invasive procedure (except a drainage tube) were other factors significantly associated with resistance to $\geqslant 1$ antibiotic.

Crude risk of resistance to an antibiotic after exposure to that same antibiotic. In univariate analysis, we did not attempt to distinguish between situations where an antibiotic had been received as a monotherapy or as part of a combination therapy (too few patients had received any specific antibiotic as part of a combination therapy). Previous exposure to ceftazidime was significantly associated with an increased risk of resistance of the bacteremic isolate toward this antibiotic (OR,

Table 2. Univariate analysis of therapies, including ceftazidime, piperacillin, imipenem, ciprofloxacin, and aminoglycosides, as risk factors for antibiotic-specific resistance in 267 bacteremic strains of *Pseudomonas aeruginosa*.

Antipseudomonal agent, included in previous therapy	Resistance of the bacteremic strain to this agent		OR (95% CI)	*P*
	Yes (cases)	No (controls)		
Ceftazidime				
Yes	3	5	—	
No	13	246	11.4 (1.6–64.7)	.008
Piperacillin[a]				
Yes	3	6	—	
No	26	231	4.4 (0.67–22.1)	.06
Imipenem[a]				
Yes	11	25	—	
No	30	186	2.7 (1.1–6.5)	.02
Ciprofloxacin				
Yes	0	9	—	
No	15	243	0.0 (0.0–9.1)	1.0
Aminoglycoside				
Yes	6	26	—	
No	37	198	1.2 (0.39–3.4)	.61

[a] One isolate was not tested against piperacillin, and 15 were not tested against imipenem.

11.4; 95% CI, 1.6–64.7; $P = .008$; table 2). Similarly, previous treatment with imipenem was significantly associated with an increased risk of resistance toward itself (OR, 2.7; 95% CI, 1.1–6.5; $P = .02$), and previous exposure to piperacillin was marginally associated with an increased risk of resistance (OR, 4.4; 95% CI, 0.67–22.1; $P = .06$). In contrast, previous exposure to ciprofloxacin or an aminoglycoside was not associated with an increased risk of resistance to themselves.

Average adjusted risk of resistance to an antibiotic after exposure to that same antibiotic. In multivariate analysis stratified for antipseudomonal agents, previous monotherapy with an antipseudomonal antibiotic was independently associated with an increased risk of subsequent resistance of the bacteremic strain to that antibiotic (OR, 2.5; 95% CI, 1.3–4.8; $P = .006$; table 3). The risk of subsequent resistance was not significantly increased among patients who had received a combination therapy (OR, 1.8; 95% CI, 0.55–5.6; $P = .34$). However, no significant difference was observed between combination therapies and monotherapies in terms of independent risk of subsequent resistance (OR, 0.70; 95% CI, 0.20–2.53; $P = .59$). Finally, severe sepsis or shock as the primary manifestation of bacteremia was marginally associated with an increased risk of resistance after controlling for previous antibiotic therapy.

DISCUSSION

For clinicians who initiate empiric treatment in a clinical situation compatible with *P. aeruginosa* bacteremia, it is crucial to assess the potential risk of a resistant causative bacterium. In this retrospective study, 80 (30%) of 267 consecutive bacteremic isolates of *P. aeruginosa* were resistant to ≥1 antipseudomonal drug. Twenty-four percent of bacteremic episodes occurred in patients who had been previously exposed to one or several of these agents. Imipenem and aminoglycosides were the most commonly administered antibiotics before the bacteremic event, and they were also the agents toward which the bacteremic isolates were the most frequently resistant. In univariate analysis, previous exposure to ceftazidime, piperacillin, and imipenem were significantly or marginally associated with an increased risk of resistance of the bacteremic isolate to themselves. After controlling for covariates, the average resistance of the bacteremic strain to an antibiotic was 2.5 times more likely when the patient had received previous monotherapy with this antibiotic that when he had not been exposed to it. No other treatment variable and none of the characteristics related to patients and hospital environment independently predicted resistance of the bacteremic isolate to an antipseudomonal drug.

Exposure to antibiotics predisposes patients to colonization with *P. aeruginosa* intrinsically resistant to these agents. *P. aeruginosa* has also the capacity to rapidly become resistant during the course of an antipseudomonal drug treatment [10]. Therefore, previous therapies increase the risk of infections with selected resistant *P. aeruginosa* isolates [7]. Moreover, through the selection of resistant strains, previous exposure to antibiotics also increases the risk for the subsequent administration of an inadequate antimicrobial treatment [11]. Finally, inadequate antibiotic treatments of *P. aeruginosa* bloodstream infections significantly increase the case-fatality rate [7, 11–13], prolong the hospital stay, and generate higher general costs [3, 14].

In this study, aminoglycosides were frequently administered

Table 3. Multivariate association, averaged across antipseudomonal agents, of previous exposure to an agent, and resistance to that same agent in 267 bacteremic strains of *Pseudomonas aeruginosa*.

Characteristic	Adjusted OR (95% CI)	P
Previous monotherapy with the agent	2.5 (1.3–4.8)	.006
Previous combination therapy including the agent	1.8 (0.55–5.6)	.34
Severe sepsis or septic shock	1.6 (0.94–2.6)	.08

NOTE. Stratified logistic regression analysis in which each episode of bacteremia contributed 5 times to the model (i.e., once per antipseudomonal agent). Variance estimates were adjusted for the resulting dependence among observations.

before the bacteremic event, and resistance toward these agents was common among the bacteremic isolates. Nevertheless, previous exposure to aminoglycosides was not associated with an increased risk of resistance. A possible explanation for this finding is that resistance toward aminoglycosides in these patients might not be due to a mechanism involving exposure to this antimicrobial class, but rather to the induction of the MexXY-OprM efflux system [15] by the exposure to other drugs. This multidrug efflux system of *P. aeruginosa* is responsible for resistance to aminoglycosides and is not only induced by exposure to aminoglycosides, but also by exposure to other classes of antibiotics such as macrolides or tetracycline [16].

The antibiotic ranking for postexposure risk of resistance differed in this study and that of Carmeli et al. [10]. In the latter work, exposure to imipenem was associated with the highest risk of resistance and ceftazidime with the lowest—findings the opposite of ours. Different study populations could explain these differences. Indeed, the study of Carmeli et al. [10] focused not only on bacteremic strains, but on both colonizing and invasive isolates from various clinical sites. In addition, patients included in the study by Carmeli et al. [10] were initially colonized with organisms susceptible to the antibiotics to which subsequent resistance was detected, and a minority of these resistant strains were proven to have emerged from the original susceptible clone. However, the study by Carmeli et al. [10] relied upon an even smaller number of resistant isolates than ours (28 vs. 81). One limitation of our work is the absence of genotyping of susceptible colonizing and resistant bacteremic strains, making it impossible to distinguish new acquisition of resistance by a previously susceptible strain from superinfection with a genetically unrelated resistant strain.

Considerable debate exists concerning the usefulness of combination therapies (usually the addition of an aminoglycosides to a β-lactam antibiotic) in order to reduce the risk of emergence of resistance in *P. aeruginosa* isolates [17–20]. In contrast to previous monotherapies, previous combination therapies did not predict subsequent resistance in this study. However neither us nor Carmeli et al. [10] found a significant difference in risk of resistance when monotherapy and combination therapies were directly compared with each other. We studied one of the largest retrospective series of *P. aeruginosa* bacteremia and had almost no missing information. Nevertheless, our study lacked power to identify differences in risk of resistance across antibiotics, as well as between monotherapy and combination therapies.

In conclusion, bacteremic events that followed exposure to antipseudomonal antibiotics were more likely to be due to resistant *P. aeruginosa* strains. Therefore, when initiating an empiric treatment for a possible *P. aeruginosa* bacteremia, clinicians should avoid previously administered antibiotics, and in

particular, they should avoid those that had been administered as monotherapies.

Acknowledgments

References

1. Diekema DJ, Pfaller MA, Jones RN, et al. Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. Clin Infect Dis **1999**; 29:595–607.
2. Kuikka A, Valtonen VV. Factors associated with improved outcome of *Pseudomonas aeruginosa* bacteremia in a Finnish university hospital. Eur J Clin Microbiol Infect Dis **1998**; 17:701–8.
3. Vidal F, Mensa J, Almela M, et al. Epidemiology and outcome of *Pseudomonas aeruginosa* bacteremia, with special emphasis on the influence of antibiotic treatment. Analysis of 189 episodes. Arch Intern Med **1996**; 156:2121–6.
4. Siegman-Igra Y, Ravona R, Primerman H, et al. *Pseudomonas aeruginosa* bacteremia: an analysis of 123 episodes, with particular emphasis on the effect of antibiotic therapy. Int J Infect Dis **1998**; 2:211–5.
5. Chatzinikolaou I, Abi-Said D, Bodey GP, et al. Recent experience with *Pseudomonas aeruginosa* bacteremia in patients with cancer: retrospective analysis of 245 episodes. Arch Intern Med **2000**; 160:501–9.
6. Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. Clin Infect Dis **1998**; 27(Suppl 1):S93–9.
7. Ibrahim EH, Sherman G, Ward S, et al. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest **2000**; 118:146–55.
8. Kiska DL, Gilligan PH. *Pseudomonas.* In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. 7th ed. Washington, DC: ASM Press, **1999**:517–25.
9. Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. 7th ed. Washington, DC: ASM Press, **1999**:1526–43.
10. Carmeli Y, Troillet N, Eliopoulos GM, et al. Emergence of antibiotic-resistant *Pseudomonas aeruginosa:* comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother **1999**; 43:1379–82.
11. Kollef MH, Sherman G, Ward S, et al. Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. Chest **1999**; 115:462–74.
12. Bisbe J, Gatell JM, Puig J, et al. *Pseudomonas aeruginosa* bacteremia: univariate and multivariate analyses of factors influencing the prognosis in 133 episodes. Rev Infect Dis **1988**; 10:629–35.
13. Leibovici L, Paul M, Poznanski O, et al. Monotherapy versus β-lactam–aminoglycoside combination treatment for gram-negative bacteremia: a prospective, observational study. Antimicrob Agents Chemother **1997**; 41:1127–33.
14. Carmeli Y, Troillet N, Karchmer AW, et al. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa.* Arch Intern Med **1999**; 159:1127–32.
15. Aires JR, Kohler T, Nikaido H, et al. Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob Agents Chemother **1999**; 43:2624–8.
16. Masuda N, Sakagawa E, Ohya S, et al. Contribution of the MexX-MexY-OprM efflux system to intrinsic resistance in *Pseudomonas aeruginosa.* Antimicrob Agents Chemother **2000**; 44:2242–6.
17. Nichols L, Maki DG. The emergence of resistance to β-lactam antibiotics during treatment of *Pseudomonas aeruginosa* lower respiratory tract infections: is combination therapy the solution? Chemioterapia **1985**; 4:102–9.
18. Bonhoeffer S, Lipsitch M, Levin BR. Evaluating treatment protocols to prevent antibiotic resistance. Proc Natl Acad Sci USA **1997**; 94: 12106–11.
19. Milatovic D, Braveny I. Development of resistance during antibiotic therapy. Eur J Clin Microbiol **1987**; 6:234–44.
20. Mouton JW. Combination therapy as a tool to prevent emergence of bacterial resistance. Infection **1999**; 27(Suppl 2):S24–8.